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PATENT

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TO ALL WHOM IT MAY CONCERN:

Be it known that WE, JEF DE BRABANDER and XIBIN LIAO, citizens of Belgium and China, respectively, whose post office addresses are 1112 Grove Drive, Lewisville, Texas 75077 and 3320 Hudnall Street, Apt. 253H, Dallas, Texas 75235, respectively, have invented an improvement in

SYNTHESIS OF PELORUSIDE A AND ANALOGS THEREOF FOR USE AS ANTITUMOR AGENTS

of which the following is a

SPECIFICATION

FIELD OF THE INVENTION

[0001] The present invention relates to the organic synthesis of a novel structural class of antimitotic compounds and anti-cancer pharmaceuticals, particularly Peloruside A and analogs thereof, having anti-proliferative activity and microtubule-stabilizing activity.

BACKGROUND OF THE INVENTION

[0002] A number of biological metabolites isolated from natural sources such as sponges, tunicates, plants and bacteria have been found to have anti-cancer activity. A novel secondary sponge metabolite, Peloruside A, was recently isolated from the marine sponge *Mycale sp.* and shown to have cytotoxic effects on mammalian cells in culture. Hood *et al.*, 2001, *Anticancer Drug Design*, 16:155-166 ("Hood 2001"). Hood 2001 disclose that Peloruside A is a polyoxygenated 16-membered ring macrolide containing a pyranose ring adjacent to a gem-

dimethyl moiety and that it has similarity to bryostatins, which have larger rings but which also contain a pyranose ring adjacent to a gem-dimethyl. Hood 2001 further disclose that Peloruside A has a different activity than brysotatin-1, namely, Peloruside A is not protein kinase C-dependent.

[0003] In another publication, Hood *et al.* disclose that Peloruside A has microtubule-stabilizing activity. Hood *et al.*, 2002, *Cancer Research* 62:3356-3360 ("Hood 2002"). Microtubule-stabilizing activity is a desired characteristic in an anti-cancer pharmaceutical, because drugs which interfere with mitosis have proven effective in the treatment of cancer. Hood 2002 show that Peloruside affects microtubule dynamics in a manner similar to paclitaxel (Taxol®). The structure described by Hood of Peloruside A is also shown in U.S. Patent Publication No. US 2002/0193423 and PCT Publication No. WO 01/10869.

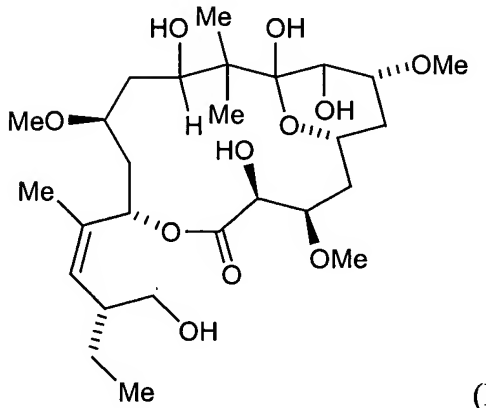
[0004] While Peloruside A has been previously described, there is no known means of synthesizing Peloruside A. Because large amounts of Peloruside A would be needed for pharmaceutical applications, a synthetic Peloruside A is desirable. The present invention describes the first synthesis of both enantiomeric forms of Peloruside A, *i.e.* (−)−Peloruside A (FIG. 1) and (+)−Peloruside A (FIG. 2) and assigns the absolute configuration of the natural product (+)−Peloruside as 2S, 3R, 5R, 7R, 8R, 9R, 11S, 13S; 15S, 18R (Peloruside numbering). The absolute configuration of natural, biologically active (+)−Peloruside A has not been assigned previously. Peloruside A has a unique architecture of a macrolactone with a very dense functionalization.

SUMMARY OF THE INVENTION

[0005] The invention includes a method of synthesis of a novel structural class of microtubule-stabilizing compounds and anti-cancer pharmaceuticals, particularly Peloruside A and analogs thereof having anti-cancer activity and microtubule-stabilizing activity similar to that of paclitaxel (Taxol®). The synthetic Pelorusides described herein have a unique architecture of a macrolactone having very dense functionalization. The present invention also documents the first case of a configuration dependent mechanistic switch for a Mitsunobu lactonization and uses a unique approach of advancing highly complex intermediates with minimal use of protecting groups. Included in the invention are the compounds, compositions containing the compounds, methods of synthesis, and methods of treatment.

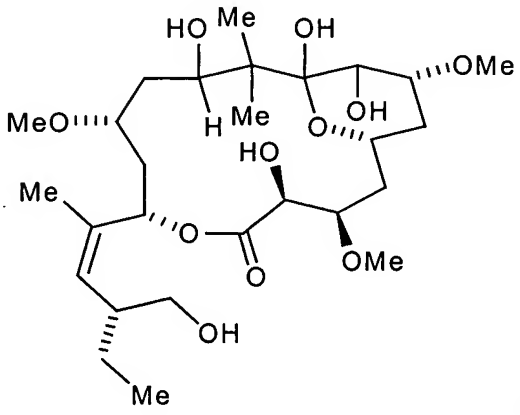
[0006] An embodiment of the invention is a synthetic compound having the ^{13}C and ^{1}H NMR signatures of FIG. 4 and FIG. 5, wherein the compound is dextrarotary, and wherein the compound has microtubule-stabilizing activity.

[0007] Another embodiment of the invention is a compound of Formula I and compositions comprising a compound of Formula I:



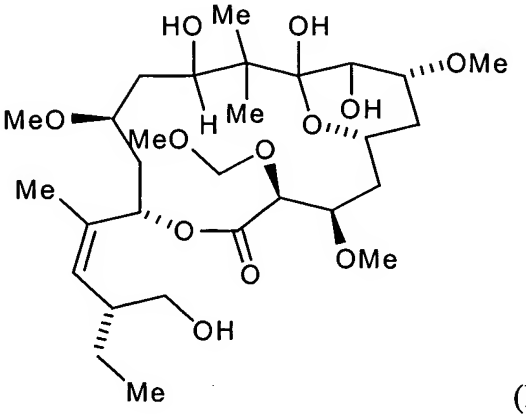
(I)

[0008] A further embodiment of the invention is a compound of the Formula II and compositions comprising a compound of Formula II:



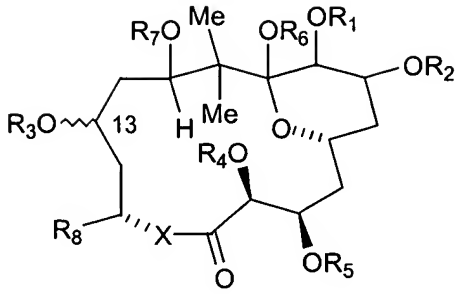
(II)

[0009] Another embodiment of the invention is a compound of the Formula III and compositions comprising a compound of Formula III:



(III)

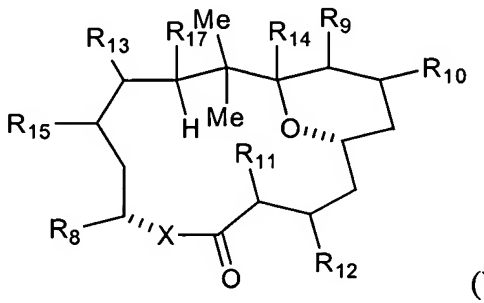
[0010] Still another embodiment of the invention is a compound of Formula IV and compositions comprising a compound of Formula IV:



(IV)

where R_1, R_2, R_3, R_4, R_5 and R_6 can be the same or different and include: H, Me, alkyl, funtionalized alkyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), and where R_8 = H, Me, aryl, heteroaryl, alkyl, functionalized alkyl, alkenyl, functionalized alkenyl, alkynyl, functionalized alkynyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl). The configuration at the carbon bearing the OR_1, OR_2, and OR_3 substituents can have the *R*- or *S*-configuration.

[0011] In still a further embodiment, the invention provides a compound of Formula V and compositions comprising a compound of Formula V:

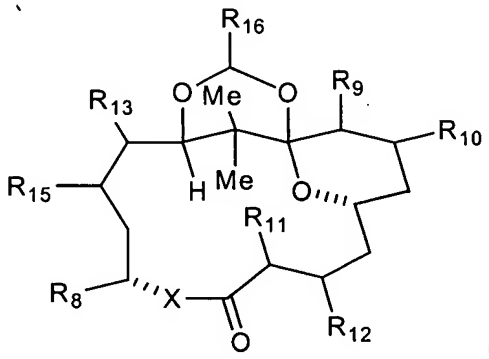


(V)

where R_{13} = H or Me, where R_{14}, R_{17} can be the same or different and include H, OH, or OR, where R_9, R_{10}, R_{11}, R_{15} can be the same or different and include H, Me, OR, where R and R_5 can be the same or different and includes H, Me, alkyl, or functionalized alkyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), where R_8 = H, Me, aryl, heteroaryl, alkyl, functionalized alkyl, alkenyl, functionalized alkenyl, alkynyl, functionalized alkynyl

(functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), and where X = O or NH. The configuration at the carbons bearing the R_9, R_{10}, R_{11}, R_{13}, R_{15} and OR_{15} substituents can be of the *R*- or *S*-configuration.

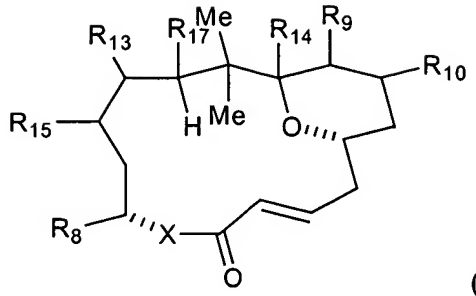
[0012] The invention also relates to a compound of Formula VI and to compositions comprising a compound of Formula VI:



(VI)

where R_{13} = H or Me, where R_9, R_{10}, R_{11}, R_{15} can be the same or different and include H, Me, OR, where R and R_5 can be the same or different and includes H, Me, alkyl, or functionalized alkyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), where R_8, R_{16} can be the same or different and include H, aryl, heteroaryl, alkyl, functionalized alkyl, alkenyl, functionalized alkenyl, alkynyl, functionalized alkynyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), and where X = O or NH. The configuration at the carbons bearing the R_9, R_{10}, R_{11}, R_{13}, R_{15}, R_{16} and OR_5 substituents can be of the *R*- or *S*-configuration.

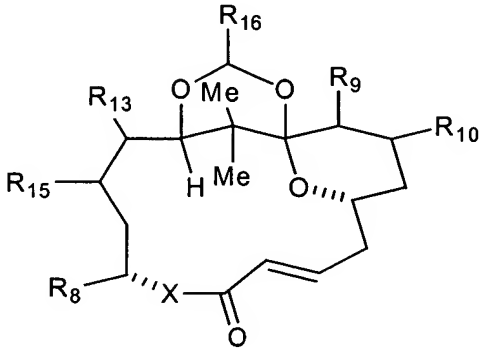
[0013] The invention also relates to a compound of Formula VII and to compositions comprising a compound of Formula VII:



(VII)

where R_{13} = H or Me, where R_{14}, R_{17} can be the same or different and include H, OH, or OR, where R_9, R_{10}, R_{15} can be the same or different and include H, Me, OR, where R includes H, Me, alkyl, or functionalized alkyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), where R_8 = H, aryl, heteroaryl, alkyl, functionalized alkyl, alkenyl, functionalized alkenyl, alkynyl, functionalized alkynyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), and where X = O or NH. The configuration at the carbons bearing the R_9, R_{10}, R_{13}, R_{15} substituents can be of the *R*- or *S*-configuration.

[0014] The invention also relates to a compound of Formula VIII and to compositions comprising a compound of Formula VIII:

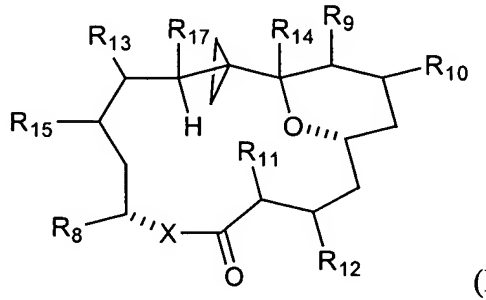


(VIII)

where R_{13} = H or Me, where R_9, R_{10}, R_{15} can be the same or different and include H, Me, OR, where R includes H, Me, alkyl, or functionalized alkyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), where R_8, R_{16} can be the same or different and include H, aryl, heteroaryl, alkyl, functionalized alkyl, alkenyl, functionalized alkenyl, alkynyl,

functionalized alkynyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), and where X = O or NH. The configuration at the carbons bearing the R_9, R_{10}, R_{13}, R_{15}, R_{16} substituents can be of the *R*- or *S*-configuration.

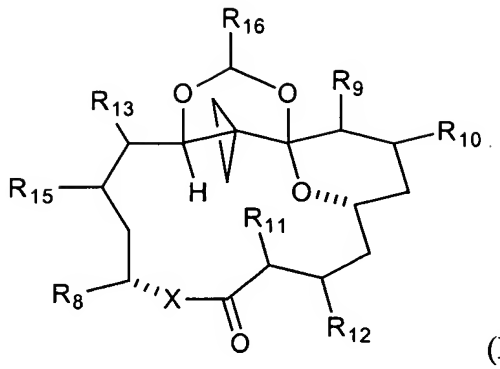
[0015] The invention also relates to a compound of Formula IX and to compositions comprising a compound of Formula IX:



(IX)

where R_{13} = H or Me, where R_{14}, R_{17} can be the same or different and include H, OH, or OR, where R_9, R_{10}, R_{11}, R_{15} can be the same or different and include H, Me, OR, where R and R_5 can be the same or different and includes H, Me, alkyl, or functionalized alkyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), where R_8 = H, aryl, heteroaryl, alkyl, functionalized alkyl, alkenyl, functionalized alkenyl, alkynyl, functionalized alkynyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), and where X = O or NH. The configuration at the carbons bearing the R_9, R_{10}, R_{11}, R_{13}, R_{15} and OR_5 substituents can be of the *R*- or *S*-configuration.

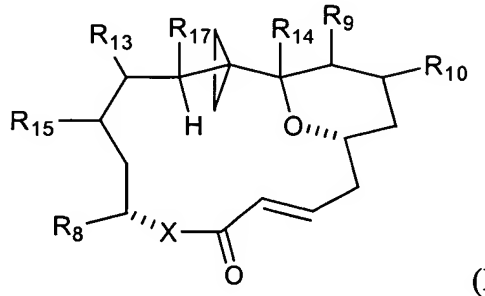
[0016] The invention also relates to a compound of Formula X and to compositions comprising a compound of Formula X:



(X)

where R_{13} = H or Me, where R_9, R_{10}, R_{11}, R_{15} can be the same or different and include H, Me, OR, where R and R_5 can be the same or different and includes H, Me, alkyl, or functionalized alkyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), where R_8, R_{16} can be the same or different and include H, aryl, heteroaryl, alkyl, functionalized alkyl, alkenyl, functionalized alkenyl, alkynyl, functionalized alkynyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), and where X = O or NH. The configuration at the carbons bearing the R_9, R_{10}, R_{11}, R_{13}, R_{15}, R_{16} and OR_5 substituents can be of the *R*- or *S*-configuration.

[0017] The invention also relates to a compound of Formula XI and to compositions comprising a compound of Formula XI:

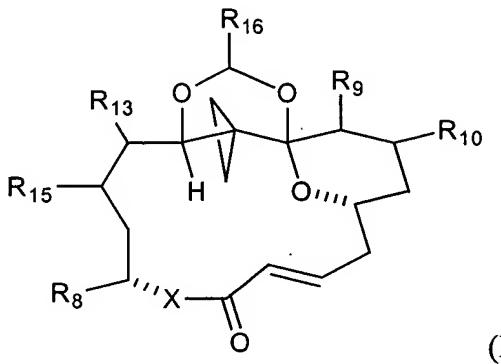


(XI)

where R_{13} = H or Me, where R_{14}, R_{17} can be the same or different and include H, OH, or OR, where R_9, R_{10}, R_{15} can be the same or different and include H, Me, OR, where R includes H, Me, alkyl, or functionalized alkyl (functionalization may include, *inter alia*, heteroatoms, halides,

aryl, heteroaryl), where R_8 = H, aryl, heteroaryl, alkyl, functionalized alkyl, alkenyl, functionalized alkenyl, alkynyl, functionalized alkynyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), and where X = O or NH. The configuration at the carbons bearing the R_9, R_{10}, R_{13}, R_{15} substituents can be of the *R*- or *S*-configuration.

[0018] The invention also relates to a compound of Formula XII and to compositions comprising a compound of Formula XII:



(XII)

where R_{13} = H or Me, where R_9, R_{10}, R_{15} can be the same or different and include H, Me, OR, where R includes H, Me, alkyl, or functionalized alkyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), where R_8, R_{16} can be the same or different and include H, aryl, heteroaryl, alkyl, functionalized alkyl, alkenyl, functionalized alkenyl, alkynyl, functionalized alkynyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), and where X = O or NH. The configuration at the carbons bearing the R_9, R_{10}, R_{13}, R_{15}, R_{16} substituents can be of the *R*- or *S*-configuration.

[0019] Yet another embodiment of the invention is a process for preparing a peloruside comprising the steps of a) synthesizing a pyran ring containing a first substituent having a carboxylic acid group and a second substituent having a hydroxyl group; and b) reacting the carboxylic acid group with the hydroxyl group to form a lactone.

[0020] The invention also provides a method of treating or preventing cancer, comprising the step of administering to a patient a therapeutically effective amount of one or more macrolactone Peloruside compounds of Formulas I through XII.

[0021] More specifically, the method for treating cancer comprises the step of contacting a tumor cell within a subject with a macrolactone peloruside of the present invention under conditions permitting the uptake of said peloruside by said tumor cell. The tumor cell may be derived from a tissue selected from the group consisting of breast, brain, lung, liver, spleen, kidney, lymph node, small intestine, blood, pancreas, colon, stomach, endometrium, prostate, testicle, ovary, skin, head, and neck, esophagus, and bone marrow. In one embodiment, the tumor cell is derived from breast and is resistant to Taxol®. In a further embodiment, the subject is human. The compounds of the present invention are also useful for suppressing the growth of tumor cells.

[0022] The invention also provides a method for stabilizing microtubule formation. The method comprises contacting microtubules with the compounds of the present invention in an amount sufficient to stabilize microtubule formation. The microtubule-stabilizing activity of the compounds of the present invention is useful, *inter alia*, for the treatment and prevention of cancer.

[0023] The invention further provides a method of regulating cell growth and proliferation in normal and malignant cells, comprising the step of administering to the cells an effective amount of a compound of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein:

FIG. 1 shows the absolute configuration of (−)−Peloruside A, the biologically inactive enantiomer according to the present invention;

FIG. 2 shows the absolute configuration of (+)−Peloruside A, the biologically active enantiomer according to the present invention;

FIG. 3 shows the strategy for synthesizing Peloruside A;

FIG. 4 is a ^{13}C NMR spectrum of synthetic Peloruside A, 75 MHz, $CDCl_3$;

FIG. 5 is a ^{1}H NMR spectrum of synthetic Peloruside A, 400 MHz, $CDCl_3$, with inserts showing magnified views of the regions from about 4.3 to about 3.6 ppm and 2.1 ppm;

FIG. 6 shows the strategy for the preparation of a C1-C13 fragment;

FIG. 7 shows the problematic glycal-epoxide solvolysis of the C1-C13 fragment;

FIG. 8 shows the solution for the problematic glycal-epoxide solvolysis for the C1-C13 fragment by elimination of the C11 sterogenic centrum;

FIG. 9 is the ^{1}H NMR signature of Ketone **6**, 400 MHz, $CDCl_3$;

FIG. 10 is the ^{13}C NMR signature of Ketone **6**, 75 MHz, $CDCl_3$;

FIG. 11 is the ^{1}H NMR signature of compound **9**, 400 MHz, $CDCl_3$;

FIG. 12 is the ^{13}C NMR signature of compound **9**, 75 MHz, $CDCl_3$;

FIG. 13 is the ^{1}H NMR signature of compound **11**, 400 MHz, $CDCl_3$;

FIG. 14 is the ^{13}C NMR signature of compound **11**, 75 MHz, $CDCl_3$;

FIG. 15 is the ^{1}H NMR signature of compound **12**, 400 MHz, $CDCl_3$;

FIG. 16 is the ^{13}C NMR signature of compound **12**, 75 MHz, $CDCl_3$;

FIG. 17 is the ^{1}H NMR signature of compound **15**, 400 MHz, $CDCl_3$;

FIG. 18 is the ^{13}C NMR signature of compound **15**, 75 MHz, $CDCl_3$;

FIG. 19 is the ^{1}H NMR signature of compound **16**, 400 MHz, $CDCl_3$;

FIG. 20 is the ^{13}C NMR signature of compound **16**, 75 MHz, $CDCl_3$;

FIG. 21 is the ^{1}H NMR signature of compound **17**, 400 MHz, $CDCl_3$;

FIG. 22 is the ^{13}C NMR signature of compound **17**, 75 MHz, $CDCl_3$;

FIG. 23 is the ^{1}H NMR signature of compound **19**, 400 MHz, $CDCl_3$;

FIG. 24 is the ^{13}C NMR signature of compound **19**, 75 MHz, $CDCl_3$;

FIG. 25 is the ^{1}H NMR signature of compound **20**, 400 MHz, $CDCl_3$;

FIG. 26 is the ^{13}C NMR signature of compound **20**, 75 MHz, $CDCl_3$;

FIG. 27 is the ^{1}H NMR signature of compound **21**, 400 MHz, $CDCl_3$;

FIG. 28 is the ^{13}C NMR signature of compound **21**, 75 MHz, $CDCl_3$;

FIG. 29 is the ^{1}H NMR signature of compound **22**, 400 MHz, $CDCl_3$;

FIG. 30 is the ^{13}C NMR signature of compound **22**, 75 MHz, $CDCl_3$;

FIG. 31 is the ^{1}H NMR signature of compound **23**, 400 MHz, $CDCl_3$;

FIG. 32 is the ^{13}C NMR signature of compound **23**, 75 MHz, $CDCl_3$;

FIG. 33 is the ^{1}H NMR signature of compound **24**, 400 MHz, $CDCl_3$;

FIG. 34 is the ^{13}C NMR signature of compound **24**, 75 MHz, $CDCl_3$;

FIG. 35 is the 1D NOE IR signature of compound **24** at 5.87 ppm, 400 MHz, $CDCl_3$;

FIG. 36 is the 1D NOE IR signature of compound **24** at 1.22 ppm, 400 MHz, $CDCl_3$;

FIG. 37 is the ^1H NMR signature of compound **29**, 400 MHz, $CDCl_3$;

FIG. 38 is the 1D NOE IR signature of compound **29** at 5.53 ppm, 400 MHz, $CDCl_3$;

FIG. 39 is the 1D NOE IR signature of compound **29** at 4.54 ppm, 400 MHz, $CDCl_3$;

FIG. 40 is the 2D NOE IR signature of compound **29**, 400 MHz, $CDCl_3$;

FIG. 41 is the ^{13}C NMR signature of compound **29**, 75 MHz, $CDCl_3$;

FIG. 42 is the ^1H NMR signature of compound **30**, 400 MHz, $CDCl_3$;

FIG. 43 is the ^{13}C NMR signature of compound **30**, 75 MHz, $CDCl_3$;

FIG. 44 is the ^1H NMR signature of compound **31**, 400 MHz, $CDCl_3$;

FIG. 45 is the ^{13}C NMR signature of compound **31**, 75 MHz, $CDCl_3$;

FIG. 46 is the ^1H NMR signature of compound **32a**, 400 MHz, $CDCl_3$;

FIG. 47 is the ^{13}C NMR signature of compound **32a**, 75 MHz, $CDCl_3$;

FIG. 48 is the ^1H NMR signature of compound **33a**, 400 MHz, $CDCl_3$;

FIG. 49 is the ^{13}C NMR signature of compound **33a**, 75 MHz, $CDCl_3$;

FIG. 50 is the ^1H NMR signature of compound **34a**, 400 MHz, $CDCl_3$;

FIG. 51 is the ^{13}C NMR signature of compound **34a**, 75 MHz, $CDCl_3$;

FIG. 52 is the ^1H NMR signature of compound **36**, 400 MHz, C_6D_6;

FIG. 53 is the 1D NOE IR signature of compound **36** at 5.74 ppm, 400 MHz, C_6D_6;

FIG. 54 is the ^{13}C NMR signature of compound **36**, 75 MHz, $CDCl_3$;

FIG. 55 is the ^1H NMR signature of compound **37**, 400 MHz, $CDCl_3$;

FIG. 56 is the ^1H NMR signature of compound **38**, 400 MHz, C_6D_6;

FIG. 57 is the ^1H NMR signature of compound **38**, 400 MHz, $CDCl_3$;

FIG. 58 is the 1D NOE IR signature of compound **38** at 6.16 ppm, 400 MHz, C_6D_6;

FIG. 59 is the ^{13}C NMR signature of compound **38**, 75 MHz, $CDCl_3$;

FIG. 60 is the ^{1}H NMR signature of compound **39**, 400 MHz, $CDCl_3$;

FIG. 61 is the ^{13}C NMR signature of compound **39**, 75 MHz, $CDCl_3$;

FIG. 62 is the ^{1}H NMR signature of compound Formula II (claim 2), 400 MHz, $CDCl_3$;

FIG. 63 is the ^{1}H NMR signature of compound Formula III (claim 3), 400 MHz, $CDCl_3$;

FIG. 64 is the ^{13}C NMR signature of compound Formula III (claim 3), 75 MHz, $CDCl_3$;

FIG. 65 is the ^{1}H NMR signature of compound **ent-40** (+)-Peloruside A, 400 MHz, $CDCl_3$;

FIG. 66 shows growth curves demonstrating the effect of Peloruside A treatment on the proliferation of tumor cell lines, MDA-MB-231, BT-549, PC-3, DU-145, LoVo, and Capan-1;

FIG. 67 shows growth curves demonstrating the effect of Peloruside A treatment on the proliferation of tumor cell lines, NCI-H460, NCI-H23, NCI-H1395, and NCI-H2887;

FIG. 68 shows growth curves demonstrating the effect of Peloruside A treatment on the proliferation of tumor cell lines, Hep-G2, SK-HEP-1, HCT-116, HCT-15, SK-MEL-28, and SK-MEL-5;

FIG. 69 shows growth curves demonstrating the effect of Peloruside A treatment on the proliferation of tumor cell lines, MiaPaCa-2. SK-OV-3, CAKI-1, and A498;

FIG. 70 shows growth curves demonstrating the effect of Taxol® treatment on the proliferation of tumor cell lines, MDA-MB-231, BT-549, PC-3, DU-145, LoVo, and Capan-1;

FIG. 71 shows growth curves demonstrating the effect of Taxol® treatment on the proliferation of tumor cell lines, NCI-H460, NCI-H23, NCI-H1395, and NCI-H2887;

FIG. 72 shows growth curves demonstrating the effect of Taxol® treatment on the proliferation of tumor cell lines, Hep-G2, SK-HEP-1, HCT-116, HCT-15, SK-MEL-28, and SK-MEL-5;

FIG. 73 shows growth curves demonstrating the effect of Taxol® treatment on the proliferation of tumor cell lines, MiaPaCa-2. SK-OV-3, CAKI-1, and A498;

FIG. 74 shows the effect of 5 μM and 10 μM Taxol® on tubulin polymerization;

FIG. 75 shows the effect of 5 μM and 10 μM Peloruside A on tubulin polymerization;

FIG. 76A shows a bar graph of Peloruside A analog, LX3111, treatment on the viability of HeLa cells at 24, 48, 72 and 110 hours of treatment at various concentrations;

FIG 76B a bar graph of Peloruside A analog, LX3111, treatment on the viability of HeLa cells at 26, 48, 73 and 106 hours of treatment at various concentrations;

FIG. 77 shows bar graph of Peloruside A analog, LX3111, treatment on the viability of HeLa cells at 48 hours of drug treatment as measured by luminescence;

FIG. 78A shows a bar graph of Peloruside A analog, LX3111, treatment on the viability of SK-MEK-5 cells at 24, 48, 72 and 110 hours of treatment at various concentrations;

FIG. 78B shows a bar graph of Peloruside A analog, LX3111, treatment on the viability of SK-MEK-5 cells at 26, 48, 73 and 106 hours of treatment at various concentrations;

FIG. 79 shows a bar graph of Peloruside A analog, LX3136, treatment on the viability of SK-MEK-5 cells at 24, 49, 70 and 107 hours of treatment at various concentrations;

FIG. 80 shows bar graphs of the cytotoxic effect of Taxol® treatment on 1A9, ptx10 and ptx22 cell growth;

FIG. 81 shows bar graphs of the cytotoxic effect of Peloruside A treatment on 1A9, ptx10 and ptx22 cell growth;

FIG. 82 shows bar graphs of the cytotoxic effect of Taxol® and Peloruside A treatment on 1A9, ptx10 and ptx22 cell growth at 20, 50 and 100nM;

FIG. 83 shows several schemes for the synthesis of various intermediates of the present invention. Compound numbers refer to schemes presented in FIGS. 84-96;

FIG. 84 shows the schemes for the synthesis of intermediates **62E** and **62G**;

FIG. 85 shows the scheme for the synthesis of intermediate **62A**;

FIG. 86 shows the scheme for the synthesis of intermediate **62C**;

FIG. 87 shows the scheme for the synthesis of intermediate **62D**;

FIG. 88 shows the scheme for the synthesis of intermediate **62I**;

FIG. 89 shows the scheme for the synthesis of intermediate **62J**;

FIG. 90 shows the schemes for the synthesis of intermediates **62F** and **62H**;

FIG. 91 shows the scheme for the synthesis of intermediate **62B**;

FIG. 92 shows the structure of intermediates **61A, 61B, 61E, 61F, 61G, 61H**;

FIG. 93 shows the structure of intermediates **62A-62J**;

FIG. 94 shows the structure of intermediates **63A-63J**;

FIG. 95 shows the structure of intermediates **64A-64J**;

FIG. 96 shows the structure of intermediates **65A-65J**;

FIG. 97 shows immunofluorescent staining of tubulin in BSC-1 (Monkey Kidney epithelial) cells treated with 200nM of Taxol® or 50nM of Peloruside A.

DETAILED DESCRIPTION OF THE INVENTION

[0025] Natural products that elicit a specific and unique biological response in mammalian cells represent valuable tools for new pharmaceuticals for the treatment for various disease states. In this context, the recent isolation of the macrolactone, Peloruside A, from the marine sponge *Mycale sp.* is noteworthy. Peloruside A represents a novel structural class of microtubule-stabilizing agents. Microtubule-stabilizing compounds can be divided into three

groups (1) the terpenoids, which include taxanes, paclitaxel (Taxol®) and taxotere, (2) the macrolides, including epothilones and laulimalides, and (3) the polyhydroxylated alkatetraene lactones, including discodermolide. Peloruside is similar to epitholones in that it is a macrolide containing a 16-membered ring.

[0026] The present invention is directed to the first synthesis of both enantiomeric forms of a compound, Peloruside A. The present invention is based on the observation that the levarotatory enantiomeric form, (−)−Peloruside A, having the 2R, 3S, 5S, 7S, 8S, 9S, 11R, 13R, 15R, 18S absolute configuration, is biologically inactive, with regard to cytotoxicity and microtubule-stabilizing activity. The present invention also establishes for the first time that the biologically active dextrarotatory enantiomeric form, (+)−Peloruside A, has the 2S, 3R, 5R, 7R, 8R, 9R, 11S, 13S, 15S, 18R absolute configuration. Through the synthesis of both enantiomeric forms of Peloruside A, the present invention discloses the absolute configuration of the naturally occurring and biologically active (+)−Peloruside A as 2S, 3R, 5R, 7R, 8R, 9R, 11S, 13S, 15S, 18R. The naturally isolated Peloruside A and both enantiomeric forms of Peloruside A synthesized by the present invention have identical NMR and Mass-spectra signatures. However, only the synthetic enantiomeric form with the 2S, 3R, 5R, 7R, 8R, 9R, 11S, 13S, 15S, 18R absolute configuration is dextrarotatory (rotates the plane of polarized light, NaD-line, in a clockwise manner) and biologically active. Accordingly, the present invention discloses a method of making synthetic (+)−Peloruside A with assigned absolute configuration, and provides the first description of the absolute configuration of the biologically active dextrarotatory enantiomer. The (−) form of Peloruside A was inactive even at 10 μM concentrations when tested against the human tumor cell lines SK-MEL-5 and HeLa, whereas

the natural Peloruside A is active at concentrations from about 4 nM to about 15 nM (see Hood *et al.*, 2001, *Anticancer Drug Design* 16:155-166).

[0027] The molecular formula, relative configuration and spectroscopic data of Peloruside A were disclosed in Northcote *et al.*, (WO 01/10869 and United States Patent Publication No. 2002/0193423), (+)–Peloruside A. The absolute configuration of natural (+)–Peloruside A, however, was not assigned in Northcote *et al.*, (WO 01/10869 and United States Patent Publication No. 2002/0193423).

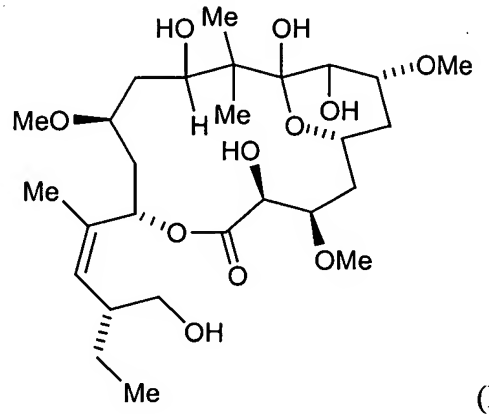
[0028] The synthetically produced Peloruside A with the 2R, 3S, 5S, 7S, 8S, 9S, 11R, 13R, 15R, 18S absolute configuration of the present invention had spectroscopic properties (^{1}H and ^{13}C NMR shown in Figs. 4 and 5; IR, HRMS) identical to the natural isolate (*see* West, Northcote and Battershill, 2000, *J. Org. Chem.* 65:445-449 for the natural isolate). However, the optical rotation of this enantiomeric form of Peloruside A with 2R, 3S, 5S, 7S, 8S, 9S, 11R, 13R, 15R, 18S absolute configuration shown in FIG. 1 was of opposite sign ($[\alpha]^{23}_{D} = -16$; c = 0.12 in CH_2Cl_2) to the one reported for the natural product ($[\alpha]^{23}_{D} = +16$; c = 0.3 in CH_2Cl_2; *see* West, Northcote and Battershill, 2000, *J. Org. Chem.* 65:445-449). The synthetically produced Peloruside A with the 2S, 3R, 5R, 7R, 8R, 9R, 11S, 13S, 15S, 18R absolute configuration of the present invention had also spectroscopic properties (^{1}H and ^{13}C NMR shown in Figs. 4 and 5; IR, HRMS) identical to the natural isolate (*see* West, Northcote and Battershill, 2000, *J. Org. Chem.* 65:445-449 for the natural isolate). This time, however, the optical rotation of this enantiomeric form of Peloruside A with 2S,3R,5R,7R,8R,9R,11S,13S,15S,18R absolute configuration shown in FIG. 2 was of identical sign ($[\alpha]^{23}_{D} = +15.5$; c = 0.2 in CH_2Cl_2) to the one reported for the natural product ($[\alpha]^{23}_{D} = +16$; c = 0.3 in CH_2Cl_2; see West, Northcote and Battershill, 2000, J.

Org. Chem. 65:445-449), which established the absolute configuration of the dextrarotatory (+)-Peloruside A as 2S,3R,5R,7R,8R,9R,11S,13S,15S,18R.

[0029] The present invention has determined the absolute configuration of (+)–Peloruside A as 2S, 3R, 5R, 7R, 8R, 9R, 11S, 13S, 15S, 18R as shown in FIG. 2. The synthesis of (–)– and *(+)*–Peloruside A is described below in Examples 1 and 2 respectively.

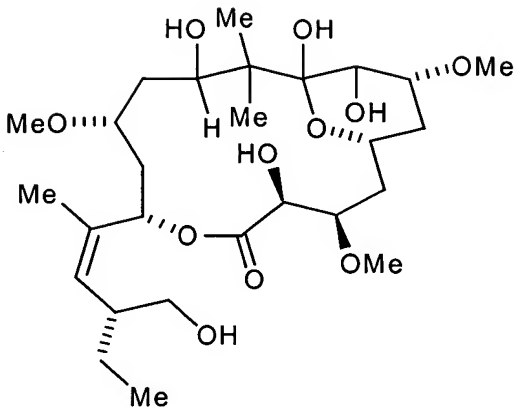
[0030] An embodiment of the invention is a synthetic compound having the NMR signatures of FIG. 4 and 5, wherein the compound is dextrarotary and wherein the compound has microtubule-stabilizing activity.

[0031] Another embodiment of the invention is a compound of Formula I and compositions comprising a compound of Formula I:



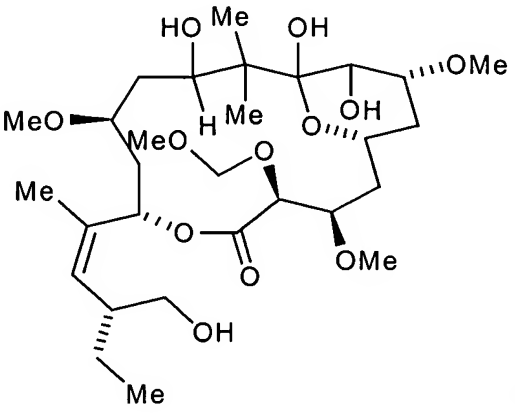
(I)

[0032] A further embodiment of the invention is a compound of the Formula II and compositions comprising a compound of Formula II:



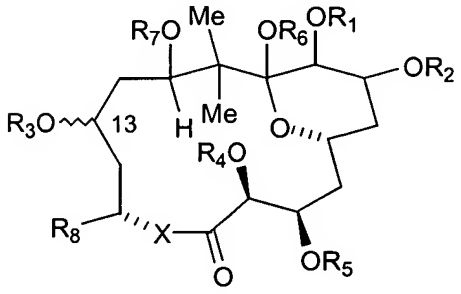
(II)

[0033] Another embodiment of the invention is a compound of the Formula III and compositions comprising a compound of Formula III:



(III)

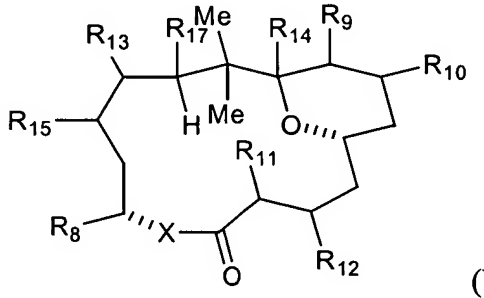
[0034] Still another embodiment of the invention is a compound of Formula IV and compositions comprising a compound of Formula IV:



(IV)

where R_1, R_2, R_3, R_4, R_5 and R_6 can be the same or different and include: H, Me, alkyl, funtionalized alkyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), and where R_8 = H, aryl, heteroaryl, alkyl, functionalized alkyl, alkenyl, functionalized alkenyl, alkynyl, functionalized alkynyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl). The configuration at the carbon bearing the OR_1, OR_2, and OR_3 substituents can have the *R*- or *S*-configuration.

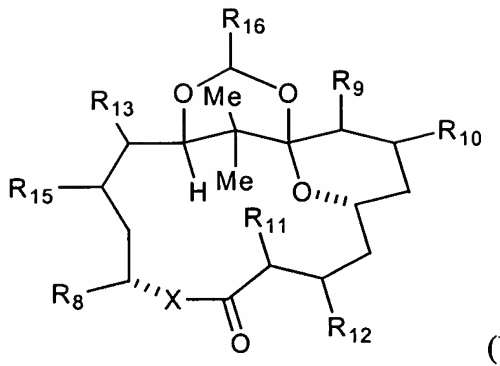
[0035] In still a further embodiment, the invention provides a compound of Formula V and compositions comprising a compound of Formula V:



(V)

where R_{13} = H or Me, where R_{14}, R_{17} can be the same or different and include H, OH, or OR, where R_9, R_{10}, R_{11}, R_{15} can be the same or different and include H, Me, OR, where R and R_5 can be the same or different and includes H, Me, alkyl, or functionalized alkyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), where R_8 = H, aryl, heteroaryl, alkyl, functionalized alkyl, alkenyl, functionalized alkenyl, alkynyl, functionalized alkynyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), and where X = O or NH. The configuration at the carbons bearing the R_9, R_{10}, R_{11}, R_{13}, R_{15} and OR_5 substituents can be of the *R*- or *S*-configuration.

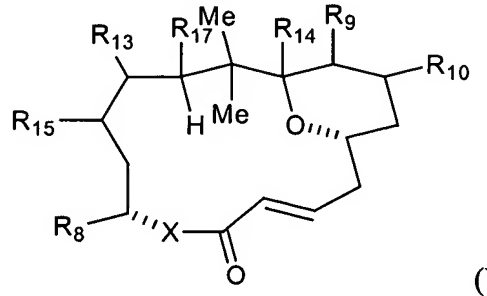
[0036] The invention also relates to a compound of Formula VI and to compositions comprising a compound of Formula VI:



(VI)

where R_{13} = H or Me, where R_9, R_{10}, R_{11}, R_{15} can be the same or different and include H, Me, OR, where R and R_5 can be the same or different and includes H, Me, alkyl, or functionalized alkyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), where R_8, R_{16} can be the same or different and include H, aryl, heteroaryl, alkyl, functionalized alkyl, alkenyl, functionalized alkenyl, alkynyl, functionalized alkynyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), and where X = O or NH. The configuration at the carbons bearing the R_9, R_{10}, R_{11}, R_{13}, R_{15}, R_{16} and OR_5 substituents can be of the *R*- or *S*-configuration.

[0037] The invention also relates to a compound of Formula VII and to compositions comprising a compound of Formula VII:

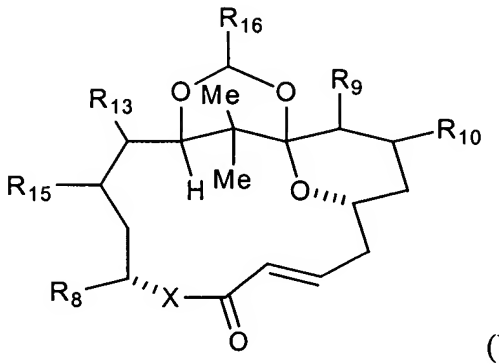


(VII)

where R_{13} = H or Me, where R_{14}, R_{17} can be the same or different and include H, OH, or OR, where R_9, R_{10}, R_{15} can be the same or different and include H, Me, OR, where R includes H, Me, alkyl, or functionalized alkyl (functionalization may include, *inter alia*, heteroatoms, halides,

aryl, heteroaryl), where R_8 = H, aryl, heteroaryl, alkyl, functionalized alkyl, alkenyl, functionalized alkenyl, alkynyl, functionalized alkynyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), and where X = O or NH. The configuration at the carbons bearing the R_9, R_{10}, R_{13}, R_{15} substituents can be of the *R*- or *S*-configuration.

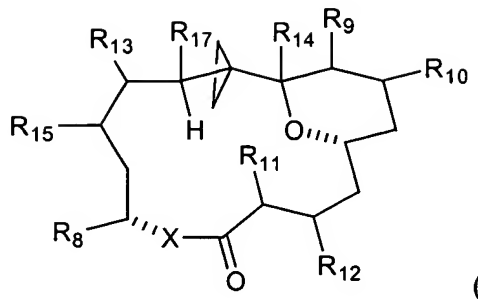
[0038] The invention also relates to a compound of Formula VIII and to compositions comprising a compound of Formula VIII:



(VIII)

where R_{13} = H or Me, where R_9, R_{10}, R_{15} can be the same or different and include H, Me, OR, where R includes H, Me, alkyl, or functionalized alkyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), where R_8, R_{16} can be the same or different and include H, aryl, heteroaryl, alkyl, functionalized alkyl, alkenyl, functionalized alkenyl, alkynyl, functionalized alkynyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), and where X = O or NH. The configuration at the carbons bearing the R_9, R_{10}, R_{13}, R_{15}, R_{16} substituents can be of the *R*- or *S*-configuration.

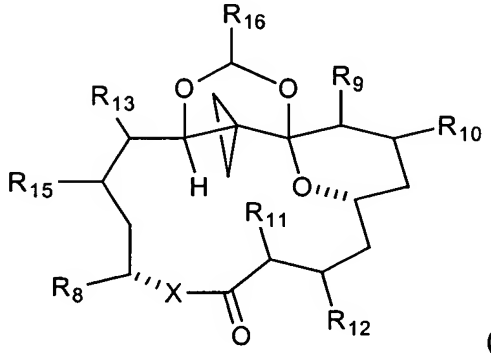
[0039] The invention also relates to a compound of Formula IX and to compositions comprising a compound of Formula IX:



(IX)

where R_{13} = H or Me, where R_{14}, R_{17} can be the same or different and include H, OH, or OR, where R_9, R_{10}, R_{11}, R_{15} can be the same or different and include H, Me, OR, where R and R_5 can be the same or different and includes H, Me, alkyl, or functionalized alkyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), where R_8 = H, aryl, heteroaryl, alkyl, functionalized alkyl, alkenyl, functionalized alkenyl, alkynyl, functionalized alkynyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), and where X = O or NH. The configuration at the carbons bearing the R_9, R_{10}, R_{11}, R_{13}, R_{15} and OR_5 substituents can be of the *R*- or *S*-configuration.

[0040] The invention also relates to a compound of Formula X and to compositions comprising a compound of Formula X:

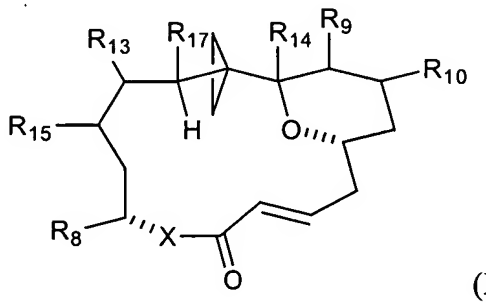


(X)

where R_{13} = H or Me, where R_9, R_{10}, R_{11}, R_{15} can be the same or different and include H, Me, OR, where R and R_5 can be the same or different and includes H, Me, alkyl, or functionalized alkyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), where R_8,

R_{16} can be the same or different and include H, aryl, heteroaryl, alkyl, functionalized alkyl, alkenyl, functionalized alkenyl, alkynyl, functionalized alkynyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), and where X = O or NH. The configuration at the carbons bearing the R_9, R_{10}, R_{11}, R_{13}, R_{15}, R_{16} and OR_5 substituents can be of the *R*- or *S*-configuration.

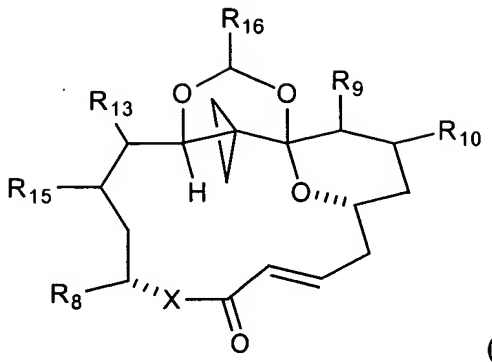
[0041] The invention also relates to a compound of Formula XI and to compositions comprising a compound of Formula XI:



(XI)

where R_{13} = H or Me, where R_{14}, R_{17} can be the same or different and include H, OH, or OR, where R_9, R_{10}, R_{15} can be the same or different and include H, Me, OR, where R includes H, Me, alkyl, or functionalized alkyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), where R_8 = H, aryl, heteroaryl, alkyl, functionalized alkyl, alkenyl, functionalized alkenyl, alkynyl, functionalized alkynyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), and where X = O or NH. The configuration at the carbons bearing the R_9, R_{10}, R_{13}, R_{15} substituents can be of the *R*- or *S*-configuration.

[0042] The invention also relates to a compound of Formula XII and to compositions comprising a compound of Formula XII:



(XII)

where R_{13} = H or Me, where R_9, R_{10}, R_{15} can be the same or different and include H, Me, OR, where R includes H, Me, alkyl, or functionalized alkyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), where R_8, R_{16} can be the same or different and include H, aryl, heteroaryl, alkyl, functionalized alkyl, alkenyl, functionalized alkenyl, alkynyl, functionalized alkynyl (functionalization may include, *inter alia*, heteroatoms, halides, aryl, heteroaryl), and where X = O or NH. The configuration at the carbons bearing the R_9, R_{10}, R_{13}, R_{15}, R_{16} substituents can be of the *R*- or *S*-configuration.

[0043] The present invention provides a process for preparing a macrolactone Peloruside comprising the steps of a) synthesizing a pyran ring containing a first substituent having a carboxylic acid group and a second substituent having a hydroxyl group; and b) reacting the carboxylic acid group with the hydroxyl group to form a macrolactone. The scheme for synthesizing the compounds of the present invention are shown in FIGS. 3 and 83.

[0044] The present invention also provides for compounds identified as intermediates in the synthesis of Peloruside A. They include the compounds **23, 31, 32a, 32b, 33a, 33b, 34a,** and **34b**, as discussed in Example 2. In addition, the present invention relates to the identification of compounds **61A, 61B, 61E, 61F, 61G, 61H, 62A-62J, 63A-63J, 64A-64J**, and **65A-65J**, as shown in FIGS. 92, 93, 94, 95 and 96, respectively.

[0045] The compounds of the present invention are useful for stabilizing microtubule formation. They are cytostatic and cytotoxic and can inhibit the growth of proliferating cells, and preferably tumor cells. Therefore, the compounds of the present invention are useful for inhibiting the growth of tumor cells for treating cancer. The compounds of the present invention possess microtubule-stabilizing activity similar to that of Taxol® and may therefore also be useful for inhibiting the growth of tumor cells which have become resistant to Taxol®.

[0046] Compounds may be tested for activity in the yeast *Saccharomyces cervisiae*. Yeast-based screening methods are fast and would allow for the rapid identification of macrolactones capable of stabilizing microtubule formation. To alleviate potential problems of drug resistance, *Δerg6* mutant strains, displaying reduced multi-drug resistance and more permeability to drugs due to more fluid membranes, can be used.

[0047] In a preferred embodiment, the temperature used for synthesis is, except where stated to be different, in a range from about –78°C to about 125°C, preferably 0°C to 90°C.

[0048] The present invention provides for pharmaceutical compositions comprising the compounds of the present invention. Aqueous pharmaceutical compositions of the present invention comprise an effective amount of a macrolactone Peloruside of the present invention or pharmaceutically acceptable salt thereof, dissolved and/or dispersed in a pharmaceutically acceptable carrier and/or aqueous medium.

[0049] As used herein, "physiologically and/or pharmaceutically acceptable carrier" includes any and/or all solvents, dispersion media, coatings, antibacterial and/or antifungal agents, isotonic and/or absorption delaying agents and/or the like. The use of such media and/or agents

for pharmaceutically active substances is well known in the art. Except insofar as any conventional media and/or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions. For human administration, preparations should meet sterility, pyrogenicity, general safety and/or purity standards as required by FDA Office of Biologics standards.

[0050] The biological material should be extensively dialyzed to remove undesired small molecular weight molecules and/or lyophilized for more ready formulation into a desired vehicle, where appropriate. The active compounds may generally be formulated for parenteral administration, *e.g.*, formulated for injection via the intravenous, intramuscular, sub-cutaneous, intralesional, and/or even intraperitoneal routes. The preparation of aqueous compositions that contain a therapeutically effective amount of the macrolactone Pelorusides of the invention or pharmaceutically acceptable salts thereof as an active component and/or ingredient will be known to those of skill in the art in light of the present disclosure. Typically, such compositions can be prepared as injectables, either as liquid solutions and/or suspensions; solid forms suitable for using to prepare solutions and/or suspensions upon the addition of a liquid prior to injection can also be prepared; and/or the preparations can also be emulsified.

[0051] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions and/or dispersions; formulations including sesame oil, peanut oil and/or aqueous propylene glycol; and/or sterile powders for the extemporaneous preparation of sterile injectable solutions and/or dispersions. In all cases the form must be sterile and/or must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and/or storage

and/or must be preserved against the contaminating action of microorganisms, such as bacteria and/or fungi.

[0052] Solutions of the active compounds as free base and/or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and/or mixtures thereof and/or in oils. Under ordinary conditions of storage and/or use, these preparations contain a preservative to prevent the growth of microorganisms.

[0053] Pelorusides of the present invention can be formulated into a composition in a neutral and/or salt form. Pharmaceutically acceptable salts, include the acid addition salts and/or which are formed with inorganic acids such as, for example, hydrochloric and/or phosphoric acids, and/or such organic acids as acetic, oxalic, tartaric, mandelic, and/or the like.

[0054] The carrier can also be a solvent and/or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and/or liquid polyethylene glycol, and/or the like), suitable mixtures thereof, and/or vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and/or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and/or antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and/or the like. In many cases, it will be preferable to include isotonic agents, for example, sugars and/or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and/or gelatin.

[0055] Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and/or in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and/or the like can also be employed.

[0056] The macrolactone pelorusides of the present invention may be formulated within a therapeutic mixture to comprise about 0.0001 to 1.0 milligrams, and/or about 0.001 to 0.1 milligrams, and/or about 0.1 to 1.0 and/or even about 10 milligrams per dose and/or so. Multiple doses can also be administered. It is believed that dosages may be similar to those used for Taxol®.

[0057] The present invention also provides kits comprising the pelorusides of the present invention or pharmaceutically acceptable salts thereof. Such kits will generally contain, in suitable container means, a pharmaceutically acceptable formulation of the Pelorusides of the present invention in a pharmaceutically acceptable formulation.

[0058] The container means will generally include at least one vial, test tube, flask, bottle, syringe and/or other container means, into which the macrocyclic lactones of the present invention formulation are placed, preferably, suitably allocated. The kits may also comprise a second container means for containing a sterile, pharmaceutically acceptable buffer and/or other diluent.

[0059] In order to increase the effectiveness of the macrolactones of the present invention, it may be desirable to combine these compositions with other agents effective in the treatment of hyperproliferative disease, such as anti-cancer agents. An "anti-cancer" agent is capable of negatively affecting cancer in a subject, for example, by killing cancer cells, inducing apoptosis

in cancer cells, reducing the growth rate of cancer cells, reducing the incidence or number of metastases, reducing tumor size, inhibiting tumor growth, reducing the blood supply to a tumor or cancer cells, promoting an immune response against cancer cells or a tumor, preventing or inhibiting the progression of cancer, or increasing the lifespan of a subject with cancer. More generally, these other compositions would be provided in a combined amount effective to kill or inhibit proliferation of the cell. This process may involve contacting the cells with the Pelorusides of the present invention and other agent(s) at the same time.

[0060] Cancer therapies may include a variety of combination therapies with both chemical and radiation based treatments. The compounds may also be used together with immunotherapy. The compounds of the present invention may also be combined with gene therapy in which a therapeutic polynucleotide is administered before, after, or at the same time as the Peloruside of the present invention. Delivery of a vector encoding one of the following gene products will have a combined anti-hyperproliferative effect on target tissues. The compounds of the present invention may further be used in combination with surgery.

[0061] It is contemplated that other agents may be used in combination with the present invention to improve the therapeutic efficacy of treatment. These additional agents include immunomodulatory agents, agents that affect the upregulation of cell surface receptors and GAP junctions, cytostatic and differentiation agents, inhibitors of cell adhesion, or agents that increase the sensitivity of the hyperproliferative cells to apoptotic inducers.

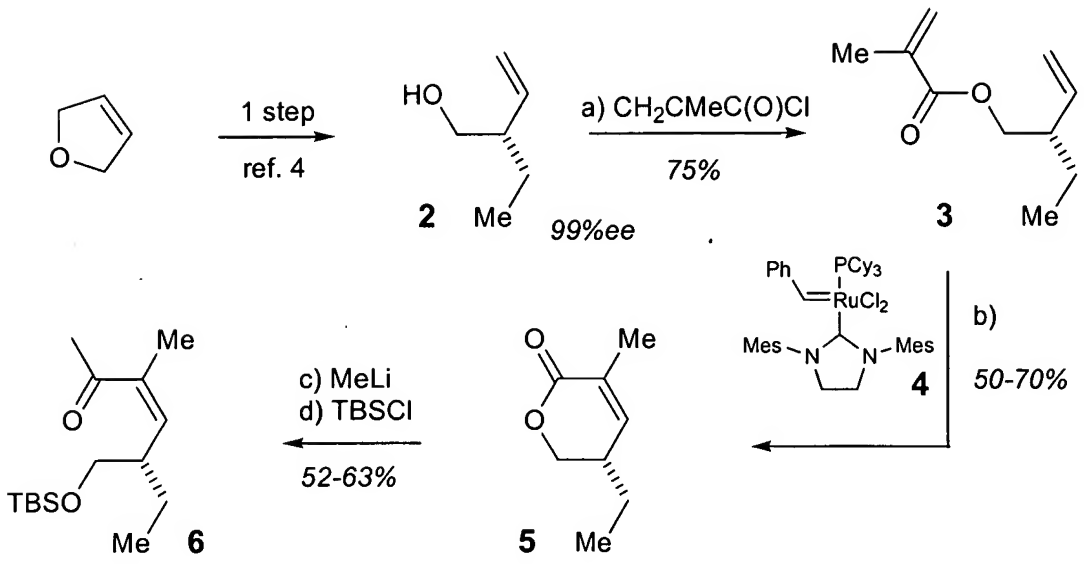
[0062] Other objects, features and advantages of the present invention will become apparent from the following specific examples. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention,

are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art.

EXAMPLES

Example 1: Synthesis of (-)-Peloruside A

[0063] Strategically, a late stage aldol coupling between a fully functionalized C1-C13 aldehyde **I** with a C14-C20 methyl ketone **II** was chosen. See FIG. 3. This approach was chosen because it was believed that access to diastereomeric seco-acids **IV** and **V**, via reduction of a common C15 ketone **III**, increases the odds for a successful macrocyclization by accessing two mechanistically distinct pathways, namely acylative or invertive macrolactonization. A concise, enantioselective synthesis of fragment **II** is outlined in Scheme 1 below. The known homoallylic alcohol **2**, prepared in enantiopure form according to the procedure described by Xu *et al.* (*J. Am. Chem. Soc.* 119:10302-10316 (1997)) was acylated with methacryloyl chloride followed by ring-closing olefin metathesis with Grubb's second generation catalyst **4**. Chatterjee *et al.*, 2000, *J. Am. Chem. Soc.* 383-3784. The resulting lactone **5** then provides a valuable entry to the (Z)-trisubstituted enone **6** by treatment with methyllithium and silylation of the primary alcohol.

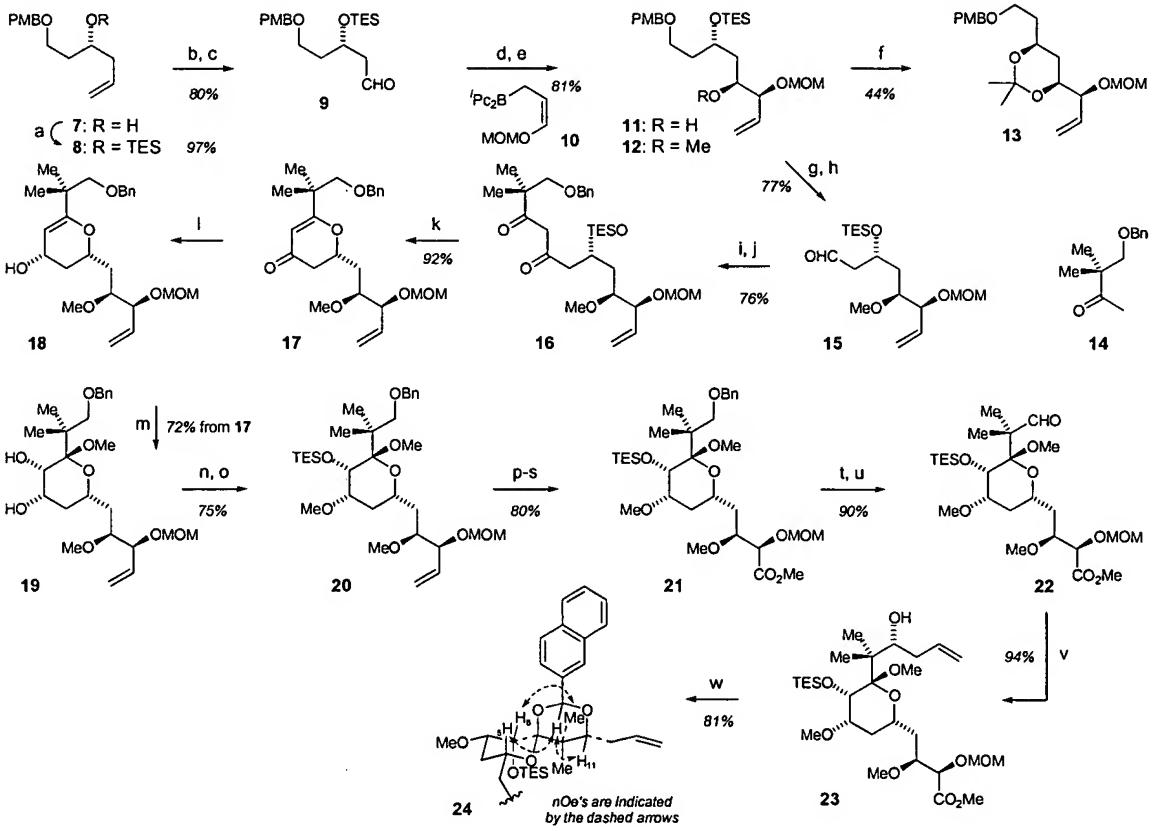


Scheme 1. Reagents and conditions: a) $CH_2CMeC(O)Cl$, iPr_2Net, DMAP, CH_2Cl_2 (75%); b) 10 mol% **4**, CH_2Cl_2 (0.0025M), reflux, 17 h (50-70% 20% dimer derived from **3**); c) MeLi, THF, −78°C; or Me_3SiCH_2Li, pentane, −78°C; d) TBSCl, imidazole, DMAP, DMF (52-63% from **5**). DMAP = 4-dimethylaminopyridine, TBS = *tert*-butyldimethylsilyl. (Ref. 4; Xu *et al.* (*J. Am. Chem. Soc.* 119:10302-10316 (1997)).

[0064] Scheme 2 below shows the approach towards the densely functionalized tetrahydropyranyl containing C1-C13 fragment. Low temperature addition of aldehyde **9** (from 7 as shown; Smith *et al.*, 2001, *J. Am. Chem. Soc.*, 123:10942-10953) to (Z)-alkoxyallylborane **10** produced the desired homoallyl alcohol diastereomer **11** (>10:1). See Brown *et al.*, 1988, *J. Am Chem. Soc.* 110:1535-1538; and Smith *et al.*, 1993, *J. Am. Chem. Soc.* 115:7612-7624. The relative 1,3-syn stereochemical relationship was demonstrated by ^{13}C NMR analysis of the acetonide derivative **13** (isopropylidene carbon resonances at 20.0, 30.3, and 98.8 ppm). *See* Rychnovsky, Rogers & Yang, 1993, *J. Org. Chem.* 58:3511-3515. Compound **11** was advanced to aldehyde **15** via methylation (→ **12**), oxidative debenzylation (DDQ), and oxidation of the

resulting primary alcohol. Addition of aldehyde **15** to a cold solution of the lithium enolate derived from **14** and Dess-Martin oxidation of the resulting aldol product fashioned β-diketone **16**. Transformation to dihydropyranone **17** ensued smoothly by stirring **16** in an acidic toluene solution (TES-deprotection / cyclodehydration). It proved critical to perform the subsequent Luche (Luche, Rodriguez-Hahn & Crabbé, 1978, *J. Chem. Soc., Chem. Commun.* 601-602) reduction at −30°C to prevent decomposition (→ **18**). Most satisfactorily, hydroxy-directed epoxidation of crude **18** followed by in situ methanolysis of the incipient glycal-epoxide produced one single glycoside **19** in high yield (72% from 17). *See*, e.g., Wender *et al.*, 1998, *J. Am. Chem. Soc.*, 120:4534-4535; and Evans *et al.*, 1999, *J. Am. Chem. Soc.* 121:7540-7552. Sequential methylation (*eq.* OH) / silylation (ax. OH) of the α-diol (→ **20**), oxidative transformation of the double bond to a C1 carboxylic acid, and diazomethane treatment furnished methyl ester **21**. Access to the C11 aldehyde **22** by hydrogenolytic removal of the benzyl ether and oxidation set the stage for progression via C11-C12 carbon bond formation. Concerns related to the feasibility of this critical bond-forming event (*see e.g.*, Wender *et al.*, 1998, *J. Am. Chem. Soc.*, 120:4534-4535; Evans *et al.*, 1999, *J. Am. Chem. Soc.* 121:7540-7552; and Kageyama *et al.*, 1990, *J. Am. Chem. Soc.* 112:7407-7408) seemed justified when various attempts to add carbon-nucleophiles to the sterically demanding aldehyde **22** met with failure. That is allylmagnesium bromide reacted competitively with the methyl ester. Lewis-acid catalyzed allyl-transfer ($allylSiMe_3$, $TiCl_4$ or BF_3Et_2O) or acetate aldol reactions (lithium or boron enloates) resulted in recovery of starting material. Ultimately, a highly efficient allyl-transfer was achieved with allyldimethylborane. Remarkably, a single alcohol diastereomer **23** was produced with C11 configuration ascertained by NOE analysis of spirocyclic ketal **24** and

advancement of **23** to Peloruside A (*vide infra*). This fortunate sterochemical outcome was not predicted *a priori*.

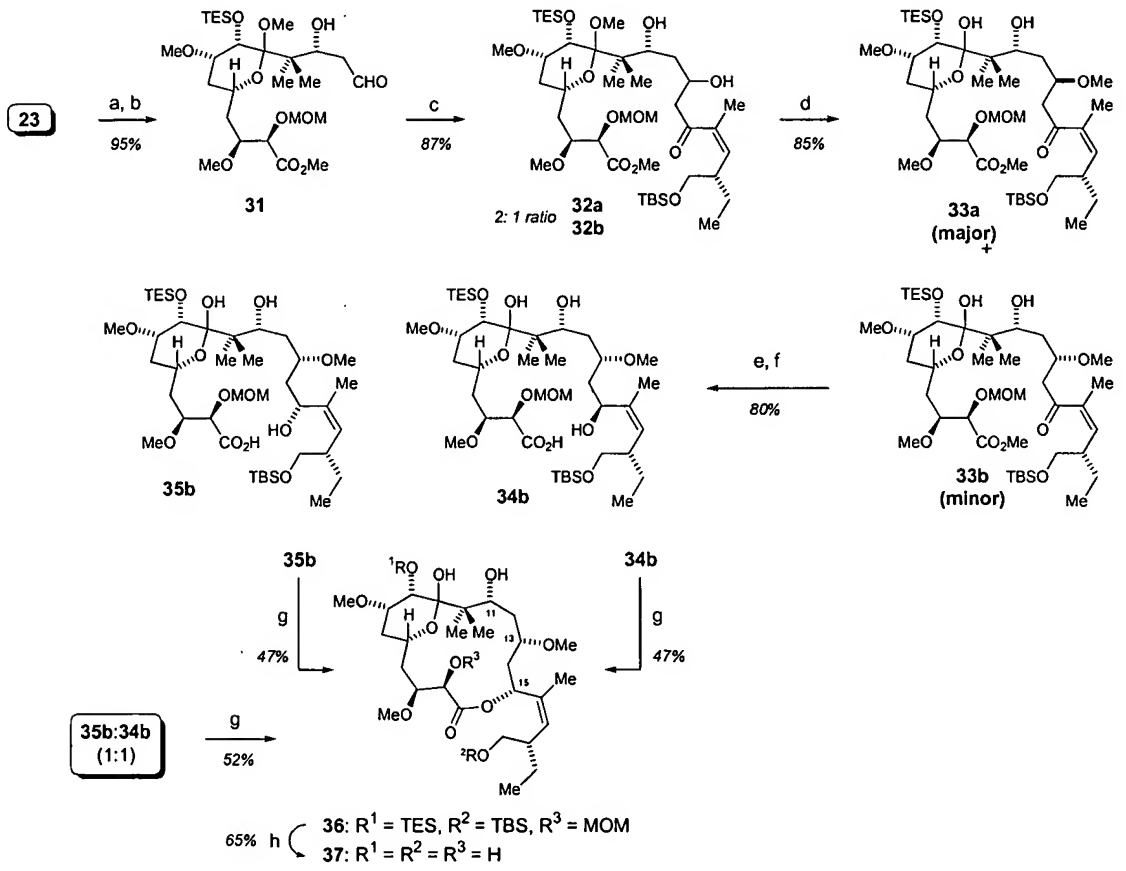


Scheme 2. Reagents and conditions: a) TESOTf, 2,6-lutidine, CH_2Cl_2 (97%); b) cat. OsO_4, NMO, acetone/H2O; c) $Pb(OAc)_4$, pyridine, CH_2Cl_2 (80% from **8**); d) **10**, (prepared from $MeOCH_2CH{=}CH_2$, sBuLi, THF, −78°C, 15 min, then (+)−Ipc_2BOMe, −78°C, 1 h, −78°C → 0°C, 1.5 h), then **9**, −95°C, 3 h, slowly warm to RT; 30% H_2O_2, NaOH, 16 h (91%); e) NaH, MeI, DMF, −5°C (89%); f) PTSA, MeOH, 20 min, remove solvent, then $Me_2C(OMe)_2$ (44%); g) DDQ, CH_2Cl_2/H_2O, 0°C (88%); h) $py{\cdot}SO_3$, Et_3N, DMSO, CH_2Cl_2, 0°C (87%); i) **14**, LDA, THF, −78°C, then **15**, −78°C (94%); j) Dess-Martin periodinane, CH_2Cl_2, −10°C (81% based on recovered starting material); k) PTSA, PhMe, RT (92%); l) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH, −30°C; m) *m*CPBA, $NaHCO_3$, CH_2Cl_2/MeOH, 0°C (72%, two steps); n) *t*BuOK, MeI, THF, 0°C; o)

TESOTf, 2,6-lutidine, CH_2Cl_2 (75% from **19**); p) cat. OsO_4, NMO, acetone/H_2O; q) $Pb(OAc)_4$, pyridine, CH_2Cl_2; r) $NaClO_2$, NaH_2PO_4, 2-Me-2-butene, *t*BuOH/H_2O; s) CH_2N_2, Et_2O, 0°C (80% from **20**); t) H_2, Pd/C (10%), MeOH (quant.); u) py·SO_3, Et_3N, DMSO, CH_2Cl_2, 0°C (90%); v) allylBEt_2, Et_2O, −10°C (94%); w) TESOTf, 2-naphthaldehyde, −78°C, then 2,6-lutidine, TESOTf, 0°C (81%). PMB = *p*-methoxybenzyl, TBS = tert-butyldimethylsilyl, TES = triethylsilyl, Tf = trifluoromethanesulfonate, NMO = 4-methylmorpholine-N-oxide, MOM = methoxymethyl, Ipc = isopinocampheyl, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, LDA = lithium diisopropylamide, PTSA = *p*-toluenesulfonic acid, *m*CPBA = *m*-chloroperbenzoic acid, Bn = benzyl, PMB = para-methoxybenzyl.

[0065] As shown in Scheme 3 below, *β*-hydroxy aldehyde **31** (from **23** as shown) was a viable partner for coupling with the enolborinate derived from **6** (Et_2BOTf, iPr_2NEt, −78°C, CH_2Cl_2). Most gratifyingly, a separable 2:1 mixture of C13 epimers **32a,b** was generated in 87% yield. At this stage, the stereochemical assignment was left unresolved, and both isomers **32a/32b** were advanced individually. Surprisingly, methylation of these compounds occurred with concomitant hydrolysis to the C9 hemiketals **33a,b**. The minor isomer **33b** was separated from the major isomer **33a** and both were advanced individually to macrocyclic lactones. Asymmetric reduction of enone **33b** using (*S*)- or (*R*)-*B*-Me-CBS-oxazaborolidine and $H_3B{\cdot}SMe_2$ (80-94% yield, ratio's > 12:1) afforded allylic alcohols **35b** and **34b** respectively. *See, e.g.*, Corey & Helal, 1998, *Angew. Chem. Int. Ed.* 37:1986-2012; and Corey & Helal, 1998, *Angew. Chem. Int. Ed.* 110:2092-2118). Note that no concomitant reduction of the C9 hemiketal was observed under these conditions. The corresponding carboxylic acids **34b/35b** were liberated quantitatively by saponification. Re-esterification with CH_2N_2 demonstrated that no

epimerization had occurred. The (Z)-configuration of the double bond was maintained throughout the synthesis and verified by NOE.

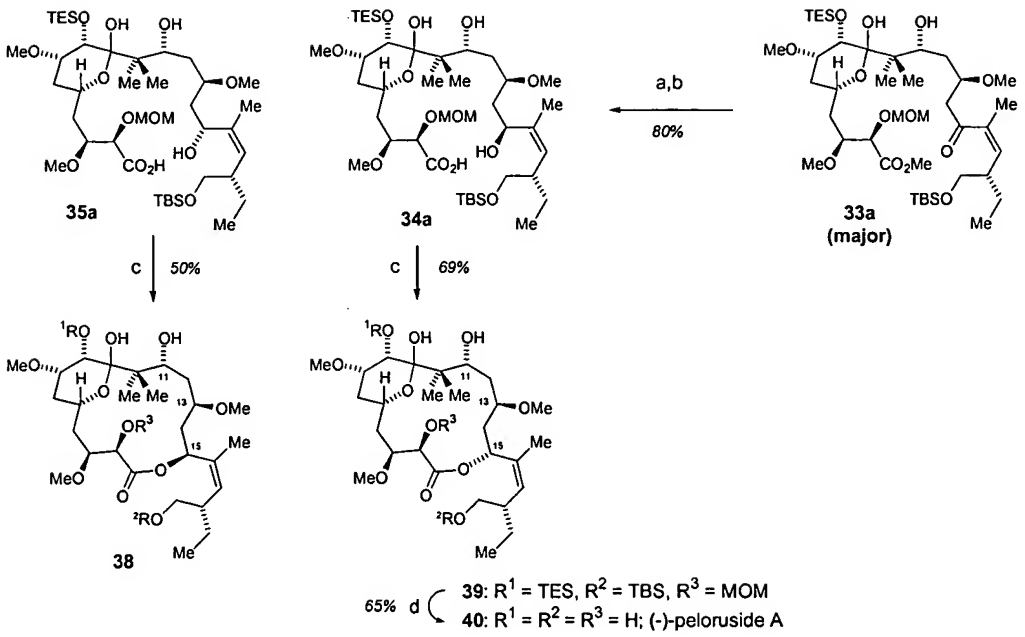


Scheme 3: Reagents and conditions: a) cat. OsO4, NMO, acetone/H_2O; b) $Pb(OAc)_4$, pyridine, CH_2Cl_2 (95% from **23**); c) 6, iPr_2Net, Et_2BOTf, CH_2Cl_2, −78°C, 15 min, −30°C, 45 min, −78°C, add **31**, −78°C, 2 h (87%, 2:1 ratio); d) Me_3OBF_4 (20 equiv.), 2,6-di-*t*Bu-4-Me-pyridine, CH_2Cl_2, RT (85%); e) 33b, (*R*)- or (*S*)-B-Me-CBS (20 equiv.), $BH_3{\cdot}SMe_2$ (7 equiv.), CH_2Cl_2, −78°C, 1 h, → RT (4 h), add MeOH (**34b**: 80%, **35b**: 80%); f) 0.3N aq. LiOH, THF, RT (quant.); g) PPh_3, DIAD, THF (0.05M), RT, 15 min, add seco-acid (0.003M in THF) via syringe pump over 2 h, then 1 h at 0°C (**36**: 47% from **34b** or **35b**, 52% from ~1:1 mixture of **34b:35b**); h) 4N HCl, THF, RT, 3 h (**37**: 65%). PMB = *p*-methoxybenzyl, TBS = tert-butyldimethylsilyl, TES =

triethylsilyl, Tf = trifluoromethanesulfonate, NMO = 4-methylmorpholine-N-oxide, MOM = methoxymethyl, CBS = catalyst named after *C*orey, *B*akshi, and *S*hibata, DIAD = diisopropylazodicarboxylate.

[0066] Both epimers **34b** and **35b** were explored as substrates for a Mitsunobu-type macrolactonization. Remarkably, the same lactone **36** was produced from either **34b**, **35b**, or an equimolar mixture of both. No diasteromeric or transposed (allylic) macrolactones were detected by NMR analysis of the non-purified mixture. This lactone was shown to have a C13 epimeric relationship to Peloruside A (*vide infra*). Clues about the sterochemistry of **36** were provided at this point by virtue of mutual NOE enhancements between the H11-H13, and H13-H15 proton pairs. This is believed to represent the first documented case of a configuration-dependent mechanistic switch for a Mitsunobu lactonization. For selected examples of Mitsunobu esterifications leading to retention of configuration when hindered alcohols are involved, *see*, Ahn & DeShong, 2002, *J. Or. Chem.* 67:1754-1759; and Smith, Safanov, & Corbett, 2002, *J. Am. Chem. Soc.* 124:11102-11113.

[0067] The structure of **36** was assigned retrospectively, *i.e.*, after the completion of (−)−Peloruside A from precursor **35a** (*vide infra*). Initial evidence for a C13 epimeric configuration surfaced however, when the ^{1}H NMR data of a globally deprotected lactone (*i.e.*, **37**), attained through the action of aqueous HCl in THF, did not match those reported for the natural product. By inference, it was realized that Peloruside A would fortuitously derive from the major aldol diastereomer **33a** (Scheme 4).



Scheme 4: Reagents and conditions: a) **33a**, (*R*)- or (*S*)-*B*-Me-CBS (20 equiv.), $BH_3 \cdot SMe_2$ (7 equiv.), CH_2Cl_2, −30°C, 1 h, → RT (4 h), add MeOH (**34a**: 80%, **35a**: 80%); b) 0.3N aq. LiOH, THF, RT (quant.); c) PPh_3, DIAD, THF (0.05M), RT, 15 min, add seco-acid (0.003M in THF) via syringe pump over 2 h, then 1 h at 0°C (**39**: 69% from **34a**); d) 4N HCl, THF, RT, 3 h (**40**: 65%). TBS = tert-butyldimethylsilyl, TES = triethylsilyl, MOM = methoxymethyl, CBS = catalyst named after Corey, Bakshi, and Shibata, DIAD = diisopropylazodicarboxylate.

[0068] Asymmetric reduction of enone **33a** using (*S*)- or (*R*)-*B*-Me-CBS-oxazaborolidine and $H_3B \cdot SMe_2$ (80-94% yield, ratio's > 12:1) afforded allylic alcohols **35a** and **34a** respectively. *See, e.g.*, Corey & Helal, 1998, *Angew. Chem. Int. Ed.* 37:1986-2012; and Corey & Helal, 1998, *Angew. Chem. Int. Ed.* 110:2092-2118). Note that no concomitant reduction of the C9 hemiketal was observed under these conditions. The corresponding carboxylic acids **34a/35a** were liberated quantitatively by saponification. Re-esterification with CH_2N_2 demonstrated that no epimerization had occurred. The (Z)-configuration of the double bond was maintained

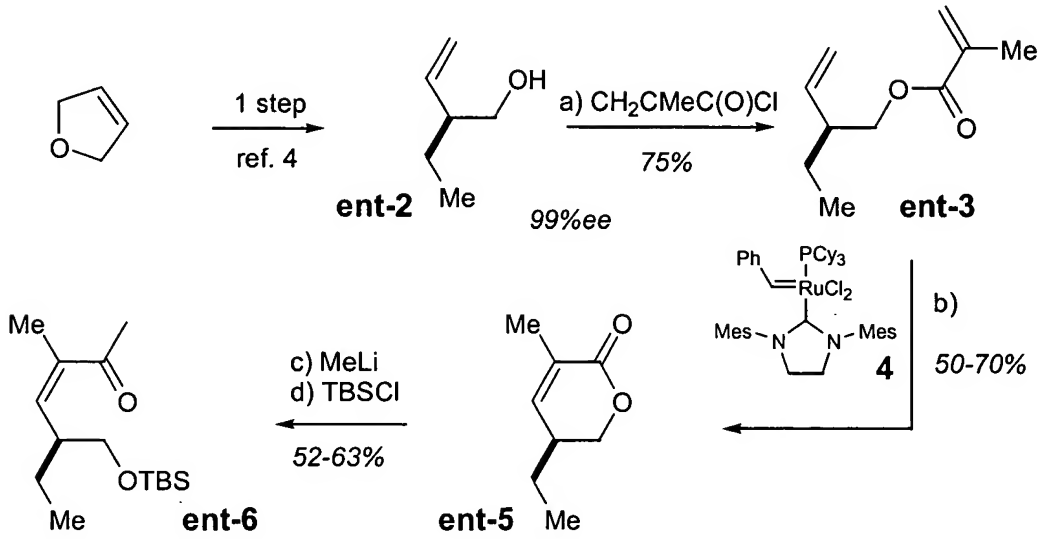
throughout the synthesis and verified by NOE. In the event, Mitsunobu lactonization of **34a** (69%) followed by simultaneous cleavage of the MOM and silyl protecting groups in lactone **39** (65%), did produce a pure compound (**40**) with spectroscopic properties (^{1}H and ^{13}C NMR, IR, HRMS) identical to the natural isolate. However, the optical rotation of synthetic Peloruside A (**40**) was of the opposite sign ($[\alpha]^{23}_D = -16$; $c = 0.12$ in CH_2Cl_2) to the one reported for the natural product ($[\alpha]^{20}\eta = -16$; $c = 0.12$ in CH_2Cl_2). Also, synthetic **40** (up to 10 μM) had no effect on the growth of cultured human tumor cell lines (SK-MEL-5, HeLa) and did not polymerize tubulin dimers into microtubule polymers. Based on these results, the absolute configuration of synthetic (−)−Peloruside A (compound **40**) was assigned as 2R, 3S, 5S, 7S, 9S, 11R, 13R, 15R, 18S. By inference, natural (+)−Peloruside A was assigned the 2S, 3R, 5R, 7R, 8R, 9R, 11S, 13S, 15S, 18R absolute configuration. This was confirmed by the synthesis of (+)−Peloruside A (see example 2 below).

Example 2. Synthesis of (+)−Peloruside A

[0069] The synthesis method described above was useful for preparing (+)− Peloruside A. The synthesis utilizes mirror image starting materials and reagents, more specifically, the mirror image of compounds **2**, **7** and **10**, *i.e.* **ent-2**, **ent-7** and **ent-10**. This synthesis was reduced to practice.

[0070] For producing (+)−Peloruside A, the homoallylic alcohol **ent-2**, prepared in enantiopure form according to the procedure described by Xu *et al.* (*J. Am. Chem. Soc.* 119:10302-10316 (1997)) was acylated with methacryloyl chloride followed by ring-closing olefin metathesis with Grubb's second generation catalyst **4** (Scheme 5). Chatterjee *et al.*, 2000, *J. Am. Chem. Soc.* 383-3784. The resulting lactone **ent-5** then provides a valuable entry to the

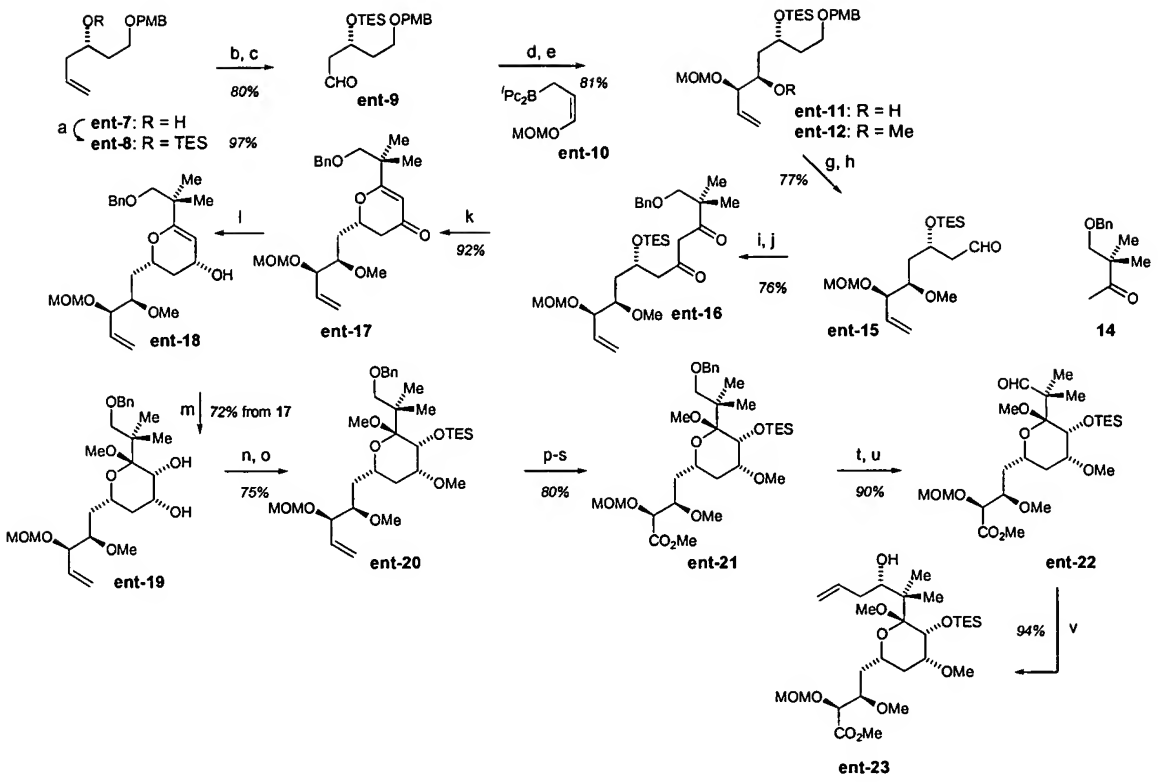
(Z)-trisubstituted enone **ent-6** by treatment with methyllithium and silylation of the primary alcohol.



Scheme 5. Reagents and conditions: a) $CH_2CMeC(O)Cl$, iPr_2Net, DMAP, CH_2Cl_2 (75%); b) 10 mol% **4**, CH_2Cl_2 (0.0025M), reflux, 17 h (50-70%); c) MeLi, THF, −78°C or Me_3SiCH_2Li, pentane, −78°C; d) TBSCl, imidazole, DMAP, DMF (52-63% from **ent-5**). DMAP = 4-dimethylaminopyridine, TBS = *tert*-butyldimethylsilyl.

[0071] Scheme 6 below shows the approach towards producing the densely functionalized tetrahydropyranyl containing C1-C13 fragment. Low temperature addition of aldehyde **ent-9** (from **ent-7** as shown; Smith *et al.*, 2001, *J. Am. Chem. Soc.*, 123:10942-10953) to (Z)-alkoxyallylborane **ent-10** produced the desired homoallyl alcohol diastereomer **ent-11** (>10:1). *See* Brown *et al.*, 1988, *J. Am. Chem. Soc.* 110:1535-1538; and Smith *et al.*, 1993, *J. Am. Chem. Soc.* 115:7612-7624. Compound **ent-11** was advanced to aldehyde **ent-15** via methylation (→ **ent-12**), oxidative debenzylation (DDQ), and oxidation of the resulting primary alcohol. Addition of aldehyde **ent-15** to a cold solution of the lithium enolate derived from **14** and Dess-

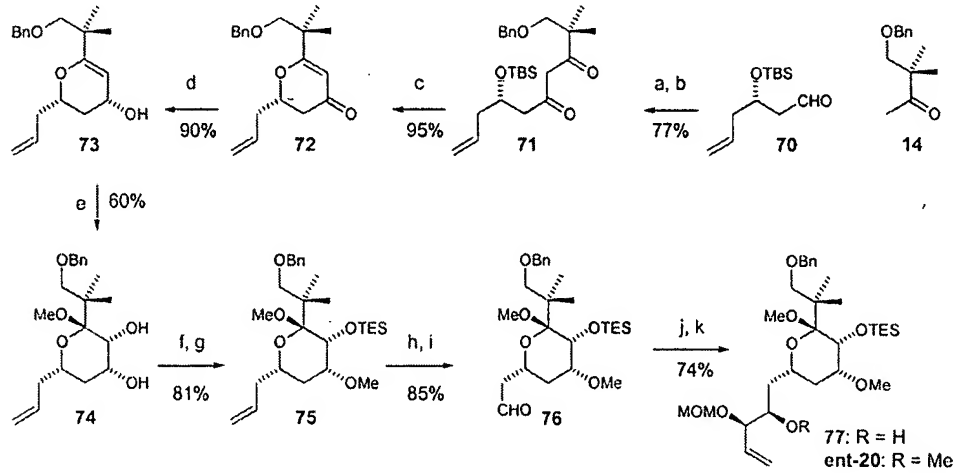
Martin oxidation of the resulting aldol product fashioned β-diketone **ent-16**. Transformation to dihydropyranone **ent-17** ensued smoothly by stirring **ent-16** in an acidic toluene solution (TES-deprotection / cyclodehydration). It proved critical to perform the subsequent Luche (Luche, Rodriguez-Hahn & Crabbé, 1978, *J. Chem. Soc., Chem. Commun.* 601-602) reduction at −30°C to prevent decomposition (→ **ent-18**). Most satisfactorily, hydroxy-directed epoxidation of crude **ent-18** followed by *in situ* methanolysis of the incipient glycal-epoxide produced one single glycoside **ent-19** in high yield (72% from **ent-17**). *See, e.g.*, Wender *et al.*, 1998, *J. Am. Chem. Soc.*, 120:4534-4535; and Evans *et al.*, 1999, *J. Am. Chem. Soc.* 121:7540-7552. Sequential methylation (*eq.* OH) / silylation (*ax.* OH) of the α-diol (→ **ent-20**), oxidative transformation of the double bond to a C1 carboxylic acid, and diazomethane treatment furnished methyl ester **ent-21**. Access to the C11 aldehyde **ent-22** by hydrogenolytic removal of the benzyl ether and oxidation set the stage for progression via C11-C12 carbon bond formation. A highly efficient allyl-transfer was achieved with allyldimethylborane. A single alcohol diastereomer **ent-23** was produced.



Scheme 6. Reagents and conditions: a) TESOTf, 2,6-lutidine, CH_2Cl_2 (97%); b) cat. OsO_4, NMO, acetone/H_2O; c) $Pb(OAc)_4$, pyridine, CH_2Cl_2 (80% from **ent-8**); d) **ent-10**, (prepared from $MeOCH_2CH{=}CH_2$, *s*BuLi, THF, −78°C, 15 min, then (−)−Ipc_2BOMe, −78°C, 1 h, −78°C, → 0°C, 1.5 h), then **ent-9**, −95°C, 3 h, slowly warm to RT; 30% H_2O_2, NaOH, 16 h (91%); e) NaH, MeI, DMF, −5°C (89%); f) PTSA, MeOH, 20 min, remove solvent, then $Me_2C(OMe)_2$ (44%); g) DDQ, CH_2Cl_2/H_2O, 0°C (88%); h) py·SO_3, Et_3N, DMSO, CH_2Cl_2, 0°C (87%); i) **14**, LDA, THF, −78°C, then **ent-15**, −78°C (94%); j) Dess-Martin periodinane, CH_2Cl_2, −10°C (81% based on recovered starting material); k) PTSA, PhMe, RT (92%); l) $NaBH_4$, $CeCl_3·7H_2O$, MeOH, −30°C; m) *m*CPBA, $NaHCO_3$, CH_2Cl_2/MeOH, 0°C (72%, two steps); n) *t*BuOK, MeI, THF, 0°C; o) TESOTf, 2,6-lutidine, CH_2Cl_2 (75% from **ent-19**); p) cat. OsO_4, NMO, acetone/H_2O; q) $Pb(OAc)_4$, pyridine, CH_2Cl_2; r) $NaClO_2$, NaH_2PO_4, 2-Me-2-butene, *t*BuOH/H_2O; s) CH_2N_2, Et_2O, 0°C (80% from **ent-20**); t) H_2, Pd/C (10%), MeOH (quant.); u)

py·SO_3, Et_3N, DMSO, CH_2Cl_2, 0°C (90%); v) allylBEt_2, Et_2O, −10°C (94%). TBS = tert-butyldimethylsilyl, PMB = *p*-methoxybenzyl, TES = triethylsilyl, NMO = 4-methylmorpholine-*N*-oxide, MOM = methoxymethyl, Ipc = isopinocampheyl, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, LDA = lithium diisopropylamide, PTSA = *p*-toluenesulfonic acid, *m*CPBA = *m*-chloroperbenzoic acid, Bn = benzyl, PMB = para-methoxybenzyl.

[0072] An alternative synthesis of compound **ent-20** is shown in Scheme 6b below. The experimental procedures for the synthesis and characterization of compounds **70-77** are provided in the experimentals section.



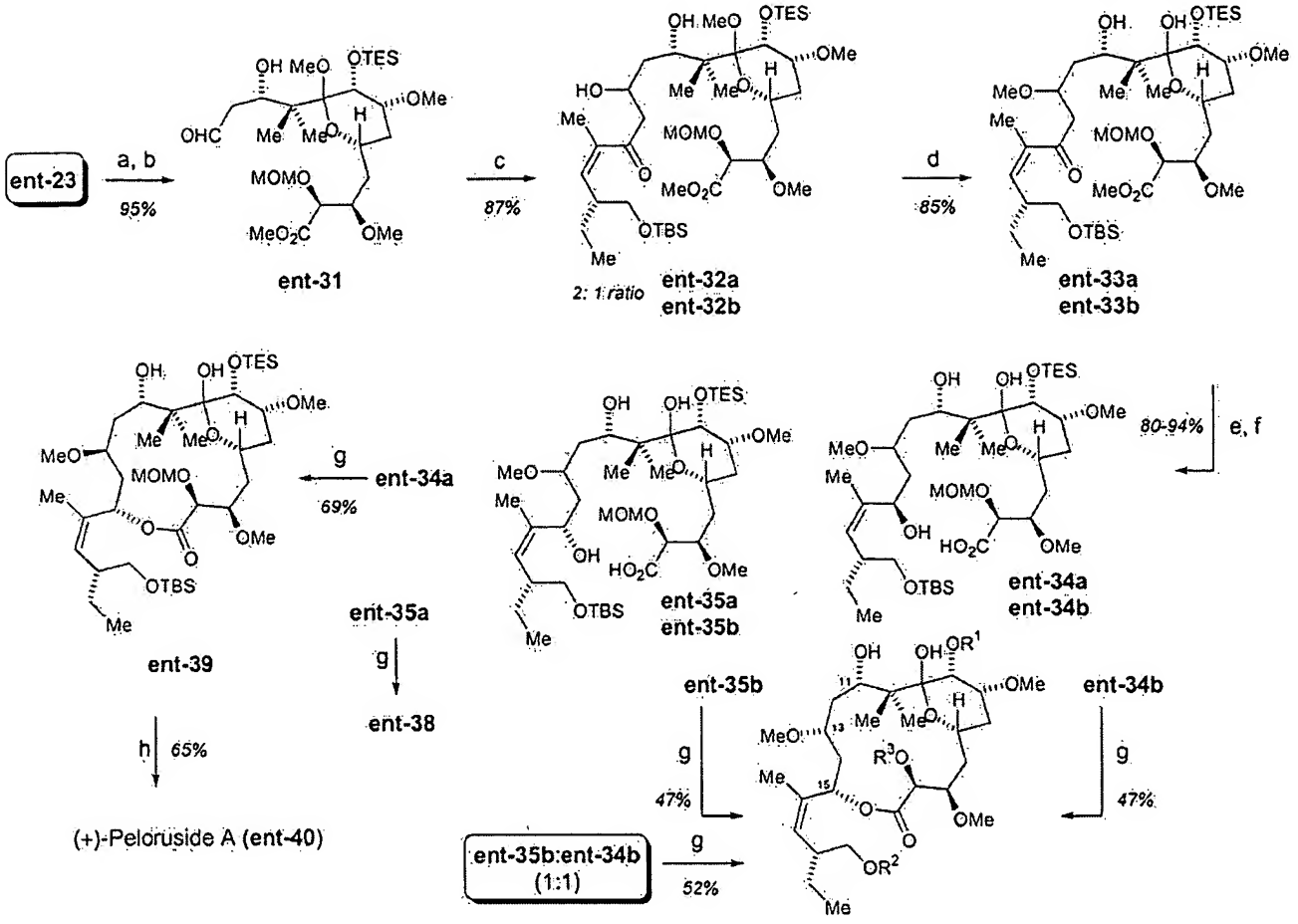
Scheme 6b. Reagents and conditions: a) **14**, LDA, THF, -78°C; then add **70**; b) SO_3.Py, DMSO, NEt_3, CH_2Cl_2 (77%); c) *p*-TsOH, MeOH (95%); d) NaBH4, $CeCl_3 \cdot 7H_2O$, MeOH (90%); e) *m*-CPBA, $NaHCO_3$, CH_2Cl_2/MeOH (60%); f) KOtBu, MeI, THF; g) TESOTf, 2,6-lutidine (81%); h) cat. OsO_4, NMO, *t*-BuOH/H_2O; i) $Pb(OAc)_4$, pyridine, CH_2Cl_2 (85%); j) **ent-10**, (prepared from $MeOCH_2CH{=}CH_2$, *s*BuLi, THF, −78°C, 15 min, then (−)-Ipc_2BOMe, −78°C, 1 h, −78°C, → 0°C, 1.5 h), then **76**, −95°C, 3 h, slowly warm to RT; 30% H_2O_2, NaOH, 16 h; e) NaH, MeI, DMF, −5°C (74%). TBS = tert-butyldimethylsilyl, TES = triethylsilyl, Tf =

trifluoromethanesulfonyl, NMO = 4-methylmorpholine-N-oxide, LDA = lithium diisopropylamide, *m*-CPBA = *m*-chloroperbenzoic acid, Bn = benzyl.

[0073] As shown in Scheme 7 below, *β*-hydroxy aldehyde **ent-31** (from **ent-23** as shown) was a viable partner for coupling with the enolborinate derived from **ent-6** (Et_2BOTf, iPr_2NEt, −78°C, CH_2Cl_2). Most gratifyingly, a separable 2:1 mixture of C13 epimers **ent-32a,b** was generated in 87% yield. Methylation of these compounds occurred with concomitant hydrolysis to the C9 hemiketals **ent-33a,b**. The minor isomer **ent-33b** was separated from the major isomer **ent-33a** and both were advanced individually to macrocyclic lactones. Asymmetric reduction of enone **ent-33b** using (*S*)- or (*R*)-*B*-Me-CBS-oxazaborolidine and $H_3B{\cdot}SMe_2$ (80-94% yield, ratio's > 12:1) afforded allylic alcohols **ent-34b** and **ent-35b** respectively. *See, e.g.*, Corey & Helal, 1998, *Angew. Chem. Int. Ed.* 37:1986-2012; and Corey & Helal, 1998, *Angew. Chem. Int. Ed.* 110:2092-2118). Note that no concomitant reduction of the C9 hemiketal was observed under these conditions. The corresponding carboxylic acids **ent-34b/ent-35b** were liberated quantitatively by saponification. Re-esterification with CH_2N_2 demonstrated that no epimerization had occurred. The (Z)-configuration of the double bond was maintained throughout the synthesis and verified by NOE. Both epimers **ent-34b** and **ent-35b** were explored as substrates for a Mitsunobu-type macrolactonization. The same lactone **ent-36** was produced from either **ent-34b**, **ent-35b**, or an equimolar mixture of both. Global deprotection of lactone **ent-36** (aq. HCl, THF) produced Peloruside analog **ent-37**. Asymmetric reduction of enone **ent-33a** using (*R*)- or (*S*)-*B*-Me-CBS-oxazaborolidine and $H_3B{\cdot}SMe_2$ (80-94% yield, ratio's > 12:1) afforded allylic alcohols **ent-35a** and **ent-34a** respectively. *See, e.g.*, Corey & Helal, 1998, *Angew. Chem. Int. Ed.* 37:1986-2012; and Corey & Helal, 1998, *Angew. Chem. Int. Ed.* 110:2092-2118). Note that no concomitant reduction of the C9 hemiketal was observed

under these conditions. The corresponding carboxylic acids **ent-34a/ent-35a** were liberated quantitatively by saponification. Re-esterification with CH_2N_2 demonstrated that no epimerization had occurred. The (Z)-configuration of the double bond was maintained throughout the synthesis and verified by NOE. In the event, Mitsunobu lactonization of **ent-34a** (69%) followed by simultaneous cleavage of the MOM and silyl protecting groups in lactone **ent-39** (65%), did produce a pure compound (**ent-40**) with spectroscopic properties (^1H and ^{13}C NMR, IR, HRMS) identical to the natural isolate. In addition, the optical rotation of synthetic Peloruside A (ent-40) was of the same sign ($[\alpha]^{23}_D = +15.5$; c = 0.2 in CH_2Cl_2) to the one reported for the natural product ($[\alpha]^{20}_D = +16$; c = 0.30 in CH_2Cl_2).

[0074] Also, synthetic **ent-40** did inhibit the growth of various cultured human tumor cell lines and did polymerize tubulin dimers into microtubule polymers. Based on these results, the absolute configuration of synthetic (+)–Peloruside A (compound **ent-40**) was assigned as 2*S*, 3*R*, 5*R*, 7*R*, 8*R*, 9*R*, 11*S*, 13*S*, 15*S*, 18*R*, fully confirming the absolute configuration of biologically active (+)–Peloruside A (**ent-40**).



Scheme 7: Reagents and conditions: a) cat. OsO_4, NMO, acetone/H_2O; b) $Pb(OAc)_4$, pyridine, CH_2Cl_2 (95% from **ent-23**); c) **ent-6**, *i*Pr_2Net, Et_2BOTf, CH_2Cl_2, –78°C, 15 min, –30°C, 45 min, –78°C, add **ent-31**, –78°C, 2 h (87%, 2:1 ratio); d) Me_3OBF_4 (20 equiv.), 2,6-di-*t*Bu-4-Me-pyridine, CH_2Cl_2, RT (85%); e) **ent-33a** or **ent-33b**, (*R*)- or (*S*)-*B*-Me-CBS (20 equiv.), $BH_3 \cdot SMe_2$ (7 equiv.), CH_2Cl_2, –30°C, 1 h, → RT (4 h), add MeOH (**ent-34a**: 80%, **ent-34b**: 80%, **ent-35a**: 94%, **ent-35b**: 80%); f) 0.3N aq. LiOH, THF, RT (quant.); g) PPh_3, DIAD, THF (0.05M), RT, 15 min, add seco-acid (0.003M in THF) via syringe pump over 2 h, then 1 h at 0°C (**ent-36**: 47% from **ent-34b** or **ent-35b**, 52% from ~1:1 mixture of **ent-34b**:**ent-35b**, **ent-38**: 69% from **ent-34a**); h) 4N HCl, THF, RT, 3 h (**ent-37**: 65%, **ent-40**: 65%).

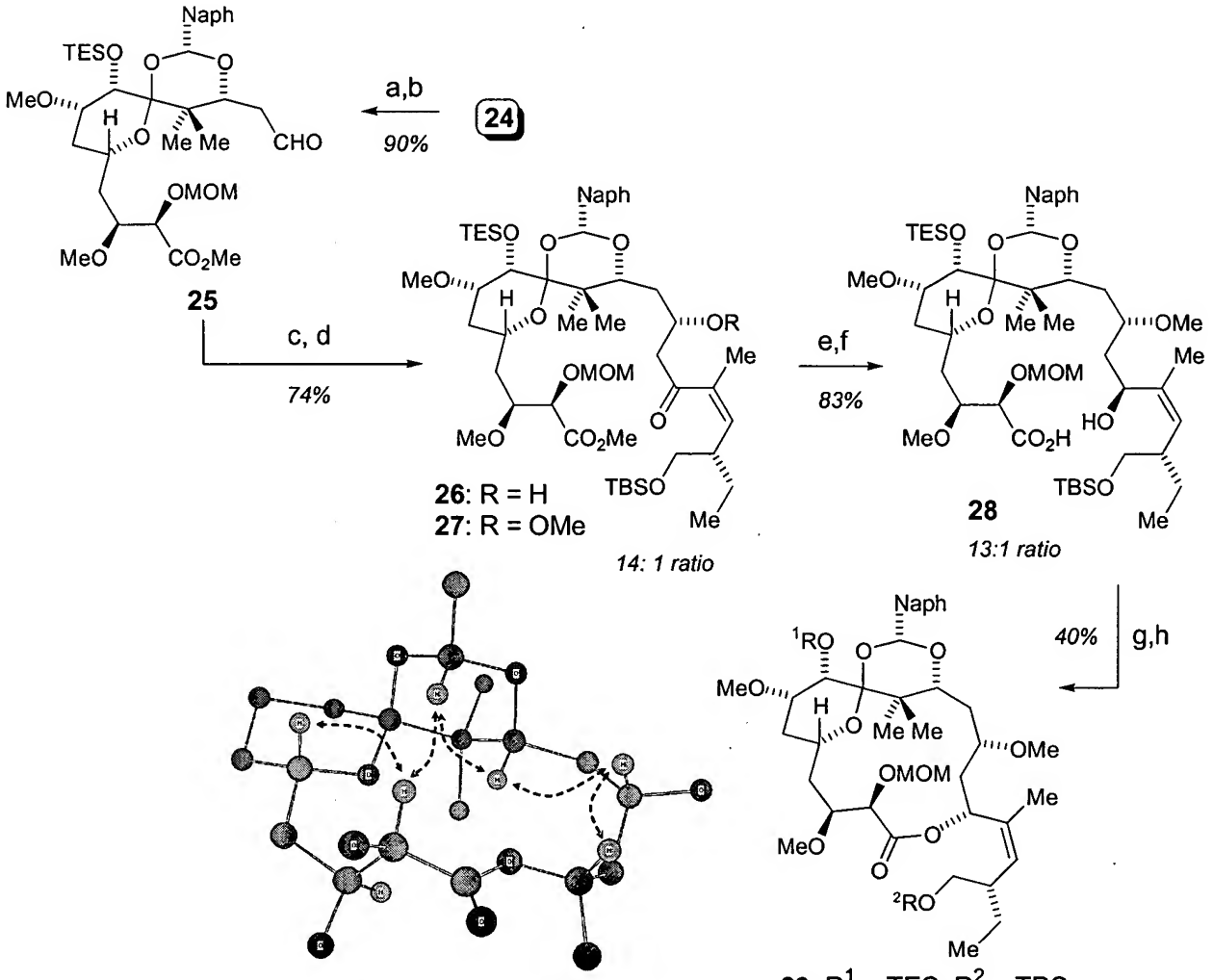
[0075] In summary, the first total synthesis of (+)−Peloruside A was accomplished. As shown above, this confirmed the absolute configuration of the dextrorotatory natural product.

Example 3. Synthesis of Peloruside Analogs

[0076] Various modifications of the synthetic routes described above in examples 1 and 2 can be readily adapted to synthesize peloruside analogs. It should be evident to skilled practicioners of the art that the syntheses of both enantiomeric series of the analogs described below are identical except for the provision that enantiomeric forms of homochiral starting materials, intermediates, reagents and catalysts are to be used for enantiomeric forms of Peloruside analogs.

[0077] An example for the synthesis of analogs with C9-C11 hydroxyls protected as a cyclic acetal is provided below. The biologically active forms of these analogs will have an enantiomeric relationship to the ones drawn in the schemes below. Union of fragments **6** and **25** and completion of a Peloruside analog is shown in Scheme 8 below. Mukayama-type aldol reaction of aldehyde **25** with the enolsilane derived from methyl ketone **6** ($BF_3 \cdot Et_2O$, CH_2Cl_2, −78°C) afforded almost exclusively (14:1) the 1,3-*syn* aldol product. This stereochemical outcome is contrary to the outcome expected from extensive literature precedents. Evans *et al.*, 1996, *J. Am. Chem. Soc.* 118:4322-4343. Evidence provided below however, indicates that aldehyde **25** entered this reaction with an unprecedented bias for the formation of the 1,3-*syn* β-hydroxy ketone **26** (80% yield). Hydroxy ketone **26** was protected as C13 methyl ether **27**, followed by CBS reduction (*see, e.g.*, Corey & Helal, 1998, *Angew. Chem. Int. Ed.* 37:1986-2012; and Corey & Helal, 1998, *Angew. Chem. Int. Ed.* 110:2092-2118) and ester hydrolysis to reach seco-acid **28**. Slow addition of this compound to a premixed solution of PPh_3 and diisopropylazodicarboxylate instigated formation of macrolactone **29** in 40-50% yield (*see, e.g.*,

Mitsunobu, 1981, *Sythesis* 1-28). The stereochemistry of **29** was deduced based on a series of NOE correlations that locate H11, H13 and H15 on the same upper side of the macrolactone ring in agreement with the assigned C13(*S*) configuration. The synthesis of **29** provides an example of Peloruside spiroacetal analogs, *i.e.* Peloruside macrocycles that have the C9 and C11 hydroxyl groups incorporated into an acetal ring, and can be prepared according to the general outline of Scheme 8. Note that enantiomeric **29**, *i.e.* **ent-29**, and analogs will be biologically active.



Chem3D-representation of **29**.
Important NOEs are illustrated with dashed arrows

Scheme 8. Reagents and conditions: a) cat. OsO_4, NMO, acetone/H_2O; b) $NaIO_4$ on silicagel, CH_2Cl_2 (90% from **24**).c) enolsilane derived from **6** (TMSOTf, Et_3N, CH_2Cl_2, −10°C, 25 min, aqueous extraction), **25**, CH_2Cl_2, −78°C, add $BF_3{\cdot}Et_2O$, 2 h (80%, 14:1 ratio); d) Me_3OBF_4, 1,8-bis(dimethylamino)naphtalene, CH_2Cl_2, RT (92%); e) (*S*)-*B*-Me-CBS (20 equiv.), $BH_3{\cdot}SMe_2$ (7 equiv.), CH_2Cl_2, −30°C, 1 h, → RT (4 h), add MeOH (83%, 13:1 ratio); f) 0.3N aq. LiOH, THF, RT (quant.); g) PPh_3, DIAD, THF (0.05M), RT, 15 min, add **28** (0.003M in THF) via syringe pump over 2 h, 1 h at RT (40-50%); h) 48% aq. HF, MeCN/H_2O, RT, 20 h (88%). MOM = methoxymethyl, Naph = 2-naphtyl, TMS = trimethylsilyl, TES = triethylsilyl, TBS = tert-butyldimethylsilyl, CBS = catalyst named after *C*orey, *B*akshi, and *S*hibata, DIAD = diisopropylazodicarboxylate, Tf = trifluoromethanesulfonyl.

[0078] Accordingly, the present invention has accomplished the first total synthesis of both enantiomeric forms of Peloruside A and documented the absolute configuration of the dextrorotatory natural product. The advancement of minimally protected intermediates was key to the successful synthesis of Peloruside A, for example, **23, 31, 32a,b, 33a,b, 34a,b**. FIGs. 3 and 6-8 further depicts the synthetic strategy of the present invention.

A. General Techniques

[0079] Unless otherwise noted, commercially available materials were used without further purification. All solvents used were of HPLC- or ACS-grade. Solvents used for moisture sensitive operations were distilled from drying agents under a nitrogen atmosphere: Et_2O and THF from sodium benzophenone ketyl; benzene and toluene from sodium; CH_2Cl_2, CH_3CN, NEt_3 and pyridine from CaH_2.

[0080] All moisture sensitive reactions were carried out under a nitrogen atmosphere with magnetic stirring. Flash chromatography (FC) was performed using *E Merck* silicagel 60 (240-400 mesh) according to the protocol of Still, Kahn, and Mitra (*J. Org. Chem.* **1978**, 43, 2923). Thin Layer chromatography was performed using precoated plates purchased from *E. Merck* (silicagel 60 PF254, 0.25 mm) that were visualized using a $KMnO_4$ or Ce (IV) stain.

[0081] Nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$, unless otherwise specified, on either a *Varian Inova-400* or *Mercury-300* spectrometer at operating frequencies of 400 / 300 MHz (^{1}H NMR) or 100 / 60 MHz (^{13}C NMR). Chemical shifts (δ) are given in ppm relative to residual solvent (usually chloroform; δ 7.27 for ^{1}H NMR or δ 77.25 for proton decoupled ^{13}C NMR), and coupling constants (*J*) in Hz. Multiplicity is tabulated as s for singlet, d for doublet, t for triplet, q for quadruplet, and m for multiplet, whereby the prefix *app* is applied in cases where the true multiplicity is unresolved, and br when the signal in question is broadened.

[0082] Infrared spectra were recorded on a *Perkin-Elmer 1000* series FTIR with wavenumbers expressed in cm^{-1} using samples prepared as thin films between salt plates. High-resolution mass spectra (HRMS) were recorded at the NIH regional mass spectrometry facility at the University of Washington, St. Louis, MO. Optical rotations were measured on a *Perkin-Elmer 241 MC* polarimeter.

B. Experimentals

Note that all the experimental procedures and spectroscopic data for compounds with an enantiomeric relationship to the ones provided below are identical, except for the optical rotations of optically active compounds, which is of opposite sign.

[0083] Ketone **6**:

[0084] $[\alpha]_D^{23}$ = +18 (*c* 0.1, $CHCl_3$); IR 2925, 1690, 1457, 1253, 1100, 836 cm^{-1}; ^1H NMR (400 MHz [FIG. 9], $CDCl_3$) δ 5.45 (1H, dq, *J* = 1.6, 10.8 Hz), 3.52 (2H, d, *J* = 6.0 Hz), 2.76 (1H, m), 2.27 (3H, s), 1.94 (3H, d, *J* = 1.6 Hz), 1.56 (1H, m), 1.23 (1H, m), 0.88 (9H, s), 0.86 (3H, t, *J* = 7.6 Hz), 0.03 (3H, s), 0.02 (3H, s) ppm; ^{13}C NMR (75 MHz [FIG. 10], $CDCl_3$) δ 203.6, 139.9, 137.1, 66.3, 43.4, 30.3, 26.0 (3C), 24.6, 21.2, 18.4, 11.8, –5.2, –5.3 ppm; HRMS Calcd for $C_{15}H_{30}O_2SiLi$ ($[M + Li]^+$): 277.2175. Found: 277.2183.

[0085] Compound **9**:

[0086] To a solution of **7** (9.5 g, 40.2 mmol) in CH_2Cl_2 (100 mL) was added 2,6–lutidine (7.1 mL, 60.3 mmol) at –65°C, followed by the drop-wise addition of TESOTf (11 mL, 48.24 mmol). The reaction mixture was slowly warmed to –45°C over 1 h, and brine (200 mL) was added. The organic layer was separated and the aq. phase was extracted with Et_2O (50 mL × 3), dried over Na_2SO_4 and concentrated. The crude intermediate **8** (15.0 g) was used for the next step without further purification. To a solution of the crude intermediate **8** (15.0 g, 40.2 mmol) in acetone (125 mL) at 0°C was added *N*-methyl-morpholine-*N*-oxide (12.5 mL, 60.3 mmol, 50% in water), followed by addition of OsO_4 (80.0 mL, 0.8 mmol, 0.1 M in BuOH). The mixture was stirred at RT for 25 h; EtOAc (50 mL) and brine (50 mL) were added, followed by Na_2SO_3 (4.00 g). After stirring for 20 min, the layers were separated and the aq. phase was extracted with EtOAc (150 mL × 3). The combined organic layers were dried over Na_2SO_4 and concentrated. The crude diol (17.0 g) was used for the next step. To a solution of diol (16 g, 38.0 mmol) in CH_2Cl_2 (160 mL) at 0°C was added pyridine (18.8 mL, 228 mmol), followed by addition of $Pb(OAc)_4$. The

resultant mixture was stirred at 0°C for 2 h. The precipitate was filtered and washed with CH_2Cl_2. The filtrate was washed with brine (200 mL), and the aq. phase was back-extracted with Et_2O (80 mL × 3). The combined organic layers were dried over Na_2SO_4 and concentrated. The residue was separated by FC (10/90/0.2 EtOAc/hexane/Et_3N) to give 8.2 g product **9** in 61% yield (three steps). **9**: $[\alpha]_D^{23} = -13.7$ (*c* 0.7, $CHCl_3$); IR: 2955, 1726, 1614, 1514, 1248, 1095, 1037, 922, 744 cm^{-1}; ^1H NMR (400MHz [FIG. 11], $CDCl_3$): 9.80 (1H, t, J = 2.2 Hz), 7.24 (2H, d, J = 8.6 Hz), 6.87 (2H, d, J = 8.6 Hz), 4.42 (1H, d, J = 11.6 Hz), 4.38 (1H, d, J = 11.6 Hz), 4.38 (1H, dd, J = 6.0, 12.0 Hz), 3.81 (3H, s), 3.51 (2H, m), 2.58 (1H, ddd, J = 2.0, 5.4, 16.0 Hz), 5.52 (1H, ddd, J = 2.0, 6.0, 16.0 Hz), 1.84 (2H, m), 0.94 (9H, t, J = 8.0 Hz), 0.60 (6H, q, J = 8.0 Hz) ppm; ^{13}C NMR (75MHz [FIG. 12], $CDCl_3$): 202.3, 159.7, 130.6, 129.5 (2C), 114.0 (2C), 72.9, 66.3, 65.8, 55.5, 51.4, 38.0, 7.0 (2C), 5.1 (2C) ppm.

[0087] Compound **11**:

[0088] To a solution of 1-(methoxymethyl)oxy-2-propene (37.2 g, 36.5 mmol) in THF (60 mL) at –78°C was added sec-BuLi (27 mL, 33.9 mmol, 1.3 M). The resulting orange yellow solution was stirred at –78°C for 25 min, and (+)-Ipc_2BOMe (11.60 g, 36.533 mmol) in THF (60 mL) was added over 10 min. After the addition was completed, the color disappeared. The mixture was stirred at –78°C for 20 min, and the cooling bath was removed. The mixture was further stirred at RT for 1.5 h and THF (40 mL) was added. The solution was cooled to –100°C, followed by addition of aldehyde **9** (8.1 g, 23 mmol) in THF (40 mL) over 15 min by double-tipped needle. The resultant mixture was stirred at –100 to –90°C for 3 h, and warmed to RT overnight. THF was removed and Et_2O (200 mL) was added. The mixture was cooled to 0°C, and 2N aq. NaOH (70 mL) was added, followed by 30% aq. H_2O_2 (40 mL) drop-wise. The

mixture was extracted with Et_2O (100 mL × 3), and the organic layers were dried over Na_2SO_4 and concentrated. The residue was purified by FC (hexane, then 15% to 20% EtOAc in hexane, 0.2% Et_3N included) to afford 9.46 g product **11** in 90.6% yield. **11:** $[\alpha]_D^{23}$ = +36.9 (*c* 1.3, $CHCl_3$); IR: 3333, 2954, 1614, 1514, 1248, 1096, 1036, 922, 743 cm^{-1}; ^1H NMR (400MHz [FIG.13], $CDCl_3$): 7.25 (2H, d, *J* = 9.0 Hz), 6.87 (2H, d, *J* = 9.0 Hz), 5.70 (1H, ddd, *J* = 7.6, 10.0, 16.6 Hz), 5.30 (1H, d, *J* = 11.0 Hz), 5.29 (1H, d, *J* = 16.6 Hz), 4.72 (1H, d, *J* = 6.8 Hz), 4.58 (1H, d, *J* = 6.8 Hz), 4.43 (1H, d, *J* = 11.6 Hz), 4.40 (1H, d, *J* = 11.6 Hz), 4.12 (1H, ddd, *J* = 6.0, 6.0, 12.0 Hz), 3.87 (1H, dd, *J* = 5.6, 7.6 Hz), 3.81 (3H, s), 3.73 (1H, dddd, *J* = 2.8, 2.8, 6.0, 8.8 Hz), 3.52 (2H, t, *J* = 6.6 Hz), 3.37 (3H, s), 3.06 (1H, d, *J* = 2.4 Hz), 1.86 (1H, ddd, *J* = 6.4, 6.4, 20.0 Hz), 1.80 (1H, ddd, *J* = 7.2, 7.2, 20.0 Hz), 1.69 (1H, dddd, *J* = 2.4, 6.4, 14.4, 20.0 Hz), 1.62 (1H, dddd, *J* = 2.8, 6.4, 14.4, 20.0 Hz), 0.95 (9H, t, *J* = 6.0 Hz), 0.61 (6H, q, *J* = 6.0 Hz) ppm; ^{13}C NMR (75MHz [FIG.14], $CDCl_3$): 159.3, 134.9, 130.7, 129.4 (2C), 119.7, 113.8 (2C), 94.1, 81.1, 72.8, 71.8, 69.1, 66.6, 55.8, 55.4, 39.8, 37.2, 7.0 (3C), 5.1 (3C) ppm; HRMS Calcd for $C_{24}H_{42}O_6SiLi$ ($[M + Li]^+$): 461.2911. Found: 461.2905.

[0089] Compound **12**:

[0090] To a solution of **11** (3.70 g, 8.14 mmol) in DMF (50 mL) at −78°C was added MeI (4.10 mL, 65.1 mmol), followed by the addition of NaH (651 mg, 16.275 mmol, 60% suspension in mineral oil) The resulting suspension was stirred at −5°C for 2 h. The mixture was poured into brine (150 mL), extracted with Et_2O (50mL × 3), and the combined organic layers were dried over Na_2SO_4 and concentrated. The residue was purified by FC (hexane, then 5% to 10% EtOAc in hexane, 0.2% Et_3N included) to give 3.4 g product **12** in 98% yield. **12**: $[\alpha]_D^{23}$ = +15.7 (*c* 2.0, $CHCl_3$); IR: 2954, 1614, 1514, 1248, 1099, 1038, 920, 743 cm^{-1}. ^1H NMR (400MHz [FIG.15],

$CDCl_3$): 7.25 (2H, d, J = 8.4 Hz), 6.87 (2H, d, J = 8.4 Hz), 5.78 (1H, ddd, J = 7.2, 10.5, 17.0 Hz), 5.27 (1H, d, J = 17.0 Hz), 5.25 (1H, d, J = 10.5 Hz), 4.68 (1H, d, J = 7.0 Hz), 4.56 (1H, d, J = 7.0 Hz), 4.43 (1H, d, J = 11.6 Hz), 4.40 (1H, d, J = 11.6 Hz), 4.07 (1H, dd, J = 4.4, 7.2 Hz), 4.02 (1H, m), 3.80 (3H, s), 3.54 (2H, t, J = 6.4 Hz), 3.41 (3H, s), 3.36 (3H, s), 3.35 (1H, m), 1.86(1H, dddd, J = 4.4, 6.8, 6.8, 13.8 Hz), 1.66–1.78 (3H, m), 0.95 (9H, t, J = 8.0 Hz), 0.58 (6H, q, J = 8.0Hz) ppm; ^{13}C NMR (75MHz [FIG. 16], $CDCl_3$): 159.2, 135.2, 130.8, 129.3 (2C), 118.6, 113.8 (2C), 94.1, 80.2, 78.3, 72.7, 67.0, 66.9, 58.7, 55.7, 55.3, 38.7, 37.2, 7.0 (3C), 5.1 (3C) ppm; HRMS Calcd for $C_{25}H_{44}O_6SiLi$ ($[M+Li]^+$): 475.3067. Found: 475.3056.

[0091] Compound **15**:

[0092] To a solution of **12** (6.9 g, 14.72 mmol) in CH_2Cl_2 (160 mL) at 0°C was added H_2O (8.9 mL), followed by addition of DDQ (4.1 g, 17.7 mmol) in one portion. The resulting mixture was stirred at 0°C for 2 h, followed by the sequential addition of Et_3N (2.0 mL), brine (200 mL) and aq. sat. $NaHCO_3$ (5 mL). The mixture was passed through celite. The aq. phase was extracted with Et_2O (100 mL × 3), and the combined organic layers were dried over $MgSO_4$ and concentrated. The residue was purified by FC (20/80/0.2 EtOAc/Hexane/Et_3N) to afford 4.5 g of product intermediate ***i*** in 88% yield. ***i***: $[\alpha]_D^{23}$ = +11 (*c* 1.4, $CHCl_3$); IR: 3336, 2954, 2880, 1463, 1153, 1100, 1034, 923, 743 cm^{-1}; ^1H NMR (400MHz, $CDCl_3$): 5.77 (1H, ddd, J = 7.2, 11.0, 17.0 Hz), 5.30 (1H, d, J = 17.0 Hz), 5.29 (1H, d, J = 11.0 Hz), 4.69 (1H, d, J = 7.0 Hz), 4.58 (1H, d, J = 7.0 Hz), 4.12 (2H, m), 3.81 (1H, m), 3.76 (1H, m), 3.44 (3H, s), 3.38 (3H, s), 3.31 (1H, ddd, J = 4.0, 4.0, 8.8 Hz), 2.65 (1H, s), 1.68–1.93 (4H, m), 0.97 (9H, t, J = 8.0 Hz), 0.62 (6H, q, J = 8.0 Hz) ppm. ^{13}C NMR (75MHz, $CDCl_3$): 134.7, 118.8, 94.2, 80.1, 78.1, 69.0, 60.1, 58.6, 55.6, 38.1, 37.8, 6.9 (3C), 5.1 (3C) ppm; HRMS Calcd for $C_{17}H_{36}O_5SiLi$ ($[M+Li]^+$): 355.2492. Found:

355.2477. To a solution of intermediate ***i*** (4.5 g, 12.91 mmol) in DMSO (30 mL) and CH_2Cl_2 (30 mL) at 0°C was added Et_3N (5.7 mL, 40.61 mmol), followed by addition of $Py{\cdot}SO_3$ (4.30 g, 27.07 mmol) in one portion. The mixture was stirred at 0°C for 1.5 h, and poured into Et_2O (100 mL) and brine (150 ml). The organic layer was separated and the aq. phase was extracted with Et_2O (50 mL × 3). The combined organic extracts were dried over $MgSO_4$ and concentrated. The residue was purified by FC (12/88/0.2 EtOAc/Hexane/Et_3N) to give 3.9 g product **15** in 87% yield. **15**: $[\alpha]_D^{23}$ = +17 (*c* 0.7, $CHCl_3$); IR: 2954, 2880 1726, 1097, 1035, 920, 743 cm^{-1}; ^1H NMR (400MHz [FIG. 17], $CDCl_3$): 9.81 (1H, t, *J* = 2.8 Hz), 5.76 (1H, ddd, *J* = 7.6, 11.0, 16.0 Hz), 5.30 (1H, d, *J* = 16.0 Hz), 5.29 (1H, d, *J* = 11.0 Hz), 4.69 (1H, d, *J* = 7.2 Hz), 4.57 (1H, d, *J* = 7.2 Hz), 4.41 (1H, ddd, *J* = 4.6, 6.8, 11.6 Hz), 4.14 (1H, dd, *J* = 4.6, 7.4 Hz), 3.43 (3H, s), 3.38 (3H, s), 3.38 (1H, m), 2.63 (1H, ddd, *J* = 2.4, 5.2, 16.0 Hz), 2.57 (1H, ddd, *J* = 3.2, 7.2, 16.0 Hz), 1.80 (1H, ddd, *J* = 4.0, 7.2, 14.4 Hz), 1.74 (1H, ddd, *J* = 4.6, 8.8, 14.4 Hz), 0.95 (9H, t, *J* = 8.0 Hz), 0.60 (6H, q, *J* = 8.0 Hz) ppm; ^{13}C NMR (75MHz [FIG. 18], $CDCl_3$): 202.3, 134.7, 119.0, 94.3, 79.6, 77.8, 65.6, 58.5, 55.8, 50.8, 38.4, 7.0 (3C), 5.0 (3C) ppm.

[0093] Compound **16**:

[0094] To a solution of diisopropylamine (2.65 mL, 18.89 mmol) in THF (30 mL) at 0°C was added n-BuLi (7.1 mL, 17.705 mmol, 2.5M in hexane). The resulting solution was stirred at 0°C for 1 h and cooled to –78°C, followed by the addition of a solution of ketone **14** (3.2 g, 15.345 mmol) in THF (15 mL) over 5 min. The resulting mixture was stirred at –78°C for 20 min, and warmed to –40°C over 35 min. After the solution was re-cooled to –78°C, a solution of aldehyde **15** (3.9 g, 11.254 mmol) in THF (15 mL) was added drop-wise over 10 min. The mixture was stirred at –78°C for 2.5 h and quenched by addition of a solution of acetic acid (1.1 mL, 18.9

mmol) in Et_2O (10 mL). Stirring was continued for 10 min, followed by pouring into brine (150 mL), extraction with Et_2O (80 mL × 3), and drying of the combined organic extracts over $MgSO_4$. After concentration, the residue was purified by FC (20/80/0.2 EtOAc/Hex/Et_3N) to give 6.0 g of product intermediate ***ii*** in 96% yield (mixture of two diastereoisomers). To a solution of intermediate ***ii*** (2.0 g, 3.62 mmol) in CH_2Cl_2 (35 mL) at –7°C was added Dess-Martin periodinane (DMP) (2.4 g, 5.43 mmol) in one portion. The resulting mixture was stirred at –7°C for 1.3 h, followed by the addition of a solution of Na_2SO_3 (5 g) in H_2O (20 mL) and sat. aq. $NaHCO_3$ (3 ml). After stirring for 10 min, the organic phase was separated and the aq. phase was extracted with Et_2O (30 mL × 3). The combined organic layers were dried over Na_2SO_4 and concentrated The residue was isolated by FC (10/90/0.2 EtOAc/Hexane/Et_3N), providing 1.0 g of product **16** and 760 mg of recovered starting material (***ii***) (81% yield based on recovered starting material). **16:** $[\alpha]_D^{23} = -5.9$ (*c* 1.5, $CHCl_3$); IR: 2955, 1603, 1460, 1098, 1031, 927, 739 cm^{-1}; ^1H NMR (400MHz [FIG. 19], $CDCl_3$) 7.24–7.36 (5H, m), 5.78 (1H, ddd, J = 7.4, 10.5, 17.5 Hz), 5.69 (1H, s), 5.28 (1H, d, J = 17.5 Hz), 5.27 (1H, d, J = 10.5 Hz), 4.69 (1H, d, J = 6.8 Hz), 4.56 (1H, d, J = 6.8 Hz), 4.51 (2H, s), 4.32 (1H, dddd, J = 4.8, 4.8, 4.8, 12.0 Hz), 4.10 (1H, dd, J = 4.8, 7.4 Hz), 3.45 (3H, s), 3.44 (2H, m), 3.40 (1H, m), 3.37 (3H, s), 2.52 (1H, dd, J = 4.8, 14.0 Hz), 2.43 (1H, dd, J = 7.6, 14.0 Hz), 1.78(1H, ddd, J = 4.0, 7.2, 14.4 Hz), 1.70 (1H, ddd, J = 4.0, 8.4, 14.4 Hz), 1.17 (6H, s), 0.92 (9H, t, J = 8.0 Hz), 0.56 (6H, q, J = 8.0 Hz) ppm; ^{13}C NMR (75MHz [FIG. 20], $CDCl_3$): 199.0, 191.9, 138.4, 134.9, 128.3 (2C), 127.5 (3C), 118.7, 98.4, 94.1, 79.8, 77.9, 76.6, 73.3, 67.3, 66.4, 58.5, 55.6, 46.8, 44.0, 38.7, 22.7, 6.9 (3C), 4.9 (3C) ppm; HRMS Calcd for $C_{30}H_{50}O_7SiLi$ ($[M+Li]^+$): 557.3486. Found: 557.3510.

[0095] Compound **17**:

[0096] To a solution of **16** (4.5 g, 8.17 mmol) in toluene (130 mL) at RT was added para-toluenesulfonic acid (233 mg, 1.225 mmol). The solution was stirred at RT for 18 h. The reaction mixture was directly transferred to column for separation by FC (35/65 EtOAc/Hexane), which afforded 3.13 g product **17** in 91% yield. **17:** $[\alpha]_D^{23}$ = +99.4 (*c* 0.65, $CHCl_3$); IR: 2889, 1667, 1598, 1457, 1338, 1098, 1033, 923, 740, 699 cm^{-1}; ^1H NMR (400MHz [FIG. 21], $CDCl_3$): 7.26–7.36 (5H, m), 5.79 (1H, ddd, *J* = 7.2, 10.8, 17.0 Hz), 5.49 (1H, s), 5.32 (1H, d, *J* = 10.6 Hz), 5.31 (1H, d, *J* = 17.0 Hz), 4.70 (1H, d, *J* = 6.6 Hz), 4.58 (1H, d, *J* = 6.6 Hz), 4.50 (2H, s), 4.50 (1H, m), 4.20 (1H, dd, *J* = 4.8, 6.8 Hz), 3.41 (3H, s), 3.34–3.41 (3H, m), 2.45 (1H, d, *J* = 11.6 Hz), 2.44 (1H, d, *J* = 5.6 Hz), 1.97 (2H, ddd, *J* = 2.8, 6.8, 6.8 Hz), 1.16 (3H, s), 1.15 (3H, s) ppm; ^{13}C NMR (75MHz [FIG. 22], $CDCl_3$): 193.4, 181.5, 138.3, 134.3, 128.4 (2C), 127.5, 127.4 (2C), 119.0, 103.1, 94.3, 79.3, 77.2, 76.9, 76.4, 73.2, 58.4, 55.6, 41.3, 41.0, 35.0, 23.0 (2C) ppm; HRMS Calcd for $C_{24}H_{34}O_6Li$ ($[M+Li]^+$): 425.2515. Found: 425.2512.

[0097] Compound **19**:

[0098] To a solution of **17** (2.3 g, 5.496 mmol) in MeOH (100 mL) was added $CeCl_3 \cdot 7H_2O$. After cooling the solution to –30°C, $NaBH_4$ (180 mg) was dded, followed by the addition of a second portion of $NaBH_4$ (120 mg) after 10 min. The mixture was stirred at –30°C for 30 min and poured into brine (100 mL) and EtOAc (50 mL). The organic phase was separated and the aq. phase was extracted with EtOAc (50 mL × 3). The combined organic layers were dried over Na_2SO_4 and concentrated. The residue **18** (1.95 g) was used for the next step. To a solution of intermediate **18** (1.95 g, 4.64 mmol) in CH_2Cl_2 (100 mL) and MeOH (50 mL) at 0°C was added $NaHCO_3$, followed by the addition of *m*-CPBA (920 mg, 5.33 mmol) in three portions every 5 min. The mixture was stirred at 0°C for 20 min and Et_3N (24 mL) was added. After 20 min, the

mixture was poured into 20 mL of Et_2O and stirring was continued for 20 min. After passing the mixture through celite, the filtrate was concentrated and the residue was purified by FC (40/60/0.4 EtOAc/Hexane/Et_3N) to give 1.57 g product **19** in 72% yield (two steps). **19:** $[\alpha]_D^{23}$ = +54.9 (*c* 0.65, $CHCl_3$); IR: 3447, 2930, 1457, 1100, 1067, 1036, 926, 737, 697 cm^{-1}; ^1H NMR (400MHz [FIG. 23], $CDCl_3$): 7.29–7.38 (5H, m), 5.83 (1H, ddd, *J* = 7.2, 11.2, 16.0 Hz), 5.38 (1H, d, *J* = 16.0 Hz), 5.37 (1H, d, *J* = 11.2 Hz), 5.00 (1H, d, *J* = 3.2 Hz), 4.71 (1H, d, *J* = 6.6 Hz), 4.58 (1H, d, *J* = 6.6 Hz), 4.55 (1H, d, *J* = 11.6 Hz), 4.51 (1H, d, *J* = 11.6 Hz), 4.17 (1H, dd, *J* = 4.4, 7.24 Hz), 3.89 (1H, m), 3.73 (1H, d, *J* = 3.2 Hz), 3.72 (1H, d, *J* = 3.2 Hz), 3.70 (1H, ddd, *J* = 3.2, 6.4, 9.0 Hz), 3.38–3.44 (2H, m), 3.42 (3H, s), 3.38 (3H, s), 3.33 (3H, s), 2.58 (1H, d, *J* = 10.8 Hz), 2.43 (1H, dd, *J* = 7.6, 14.0 Hz), 1.80 (2H, t, *J* = 6.0 Hz), 1.69 (1H, ddd, *J* = 3.0, 4.4, 12.0 Hz), 1.60 (1H, m), 1.12 (3H, s), 1.02 (3H, s) ppm; ^{13}C NMR (75MHz [FIG. 24], $CDCl_3$): 137.1, 135.1, 128.7 (2C), 128.1, 127.9 (2C), 118.7, 103.6, 94.2, 80.1, 77.8, 77.1, 73.8, 69.3, 68.6, 67.0, 58.4, 55.7, 52.0, 45.4, 36.3, 33.9, 22.8, 22.3 ppm; HRMS Calcd for $C_{25}H_{40}O_8Li$ ($[M+Li]^+$): 475.2883. Found: 475.2881.

[0100] Compound **20**:

[0101] To a solution of **19** (1.28 g, 1.28 mmol) in THF (17 mL) at 0°C was added $KOBu^t$ (182 mg, 1.54 mmol) in one portion. The resulting orange solution was stirred at 0°C for 7 min and a solution of MeI (1.61 mL, 2.561 mmol) in THF (84 mL) was added drop-wise. The resulting suspension was stirred at 0°C for 30 min, and 0.5 mL Et_3N was added followed by brine (50 mL) and Et_2O (30 mL). The aq. phase was extracted with EtOAc (30 mL × 3), and the combined organic layers were dried over Na_2SO_4 and concentrated. The residue was isolated by FC (35/65/0.3 to 45/55/0.2 EtOAc/Hexane/Et_3N) to afford 325 mg product (intermediate ***iii***) and 180

mg recovered starting material **19** in 75% yield (based on recovered starting material).

Intermediate *iii*: $[\alpha]_D^{23}$ = +42.8 (*c* 0.8, $CHCl_3$); IR: 3480, 2930, 1457, 1100, 1067, 927, 740, 700 cm^{-1}; ^1H NMR (400MHz, $CDCl_3$): 7.27–7.35 (5H, m), 5.83 (1H, ddd, *J* = 7.4, 11.2, 16.4 Hz), 5.28 (1H, d, *J* = 16.4 Hz), 5.27 (1H, d, *J* = 11.2 Hz), 4.70 (1H, d, *J* = 7.2 Hz), 4.58 (1H, d, *J* = 7.2 Hz), 4.53 (2H, s), 4.20 (1H, d, *J* = 2.0 Hz), 4.17 (1H, dd, *J* = 4.8, 6.8 Hz), 3.93 (1H, t, *J* = 2.0 Hz), 3.72 (1H, dddd, *J* = 2.8, 2.8, 6.0, 8.8 Hz), 3.58 (2H, m), 3.44 (1H, m), 3.40 (3H, s), 3.39 (3H, s), 3.38 (3H, s), 3.34 (3H, s), 3.33 (1H, m), 1.82 (1H, t, *J* = 6.0 Hz), 1.76 (1H, q, *J* = 12.0 Hz), 1.66 (1H, td, *J* = 3.2, 12.0 Hz), 1.11 (3H, s), 1.08 (3H, s) ppm; ^{13}C NMR (75MHz, $CDCl_3$): 137.9, 135.2, 128.6 (2C), 127.9 (3C), 118.8, 104.1, 94.3, 80.1, 77.9, 76.7, 76.6, 73.7, 68.9, 66.9, 58.4, 55.8, 55.7, 52.0, 45.8, 36.3, 30.2, 22.7, 21.9 ppm; HRMS Calcd for $C_{26}H_{42}O_8Li$ ($[M+Li]^+$): 489.3040. Found: 489.3024. To a solution of the alcohol intermediate ***iii*** (1.2 g, 2.487 mmol) in dry THF at -78°C was added 2,6-lutidine (731 μL, 6.22 mmol), followed by TESOTf (859 μL, 3.73 mmol). The solution was stirred at the same temperature for 40 min, followed by dilution with Et_2O (300 mL) and sat. aq. NH_4Cl (100 mL). The organic layer was separated and washed with 100 mL sat. aq. NH_4Cl, dried over Na_2SO_4 and concentrated. The residue was purified by FC (EtOAc/hexane 12/88) to provide the product **20** (1.2 g, 81%). $[\alpha]_D^{23}$ = +32 (*c* 0.5, $CHCl_3$) {**ent-20**: $[\alpha]_D^{23}$ = –24 (*c* 0.5, $CHCl_3$)}; IR 2947, 1459, 1099, 1039, 740 cm^{-1}; ^1H NMR (400 MHz [FIG. 25], $CDCl_3$) δ 7.31 (5H, m), 5.83 (1H, dddd, *J* = 7.2, 7.6, 10.0, 17.2 Hz), 5.26 (2H, m), 4.70 (1H, d, *J* = 6.4 Hz), 4.58 (1H, d, *J* = 6.4 Hz), 4.53 (1H, d, *J* = 12.4 Hz), 4.42 (1H, d, *J* = 12.4 Hz), 4.16 (1H. dd, *J* = 4.4, 7.2 Hz), 3.93 (1H, d, *J* = 1.6 Hz), 3.78 (1H, d, *J* = 8.8 Hz), 3.67 (1H, m), 3.47 (1H, m), 3.38 (3H, s), 3.37 (3H, s), 3.34 (1H, d, *J* = 8.8 Hz), 3.33 (3H, s), 3.32 (3H, s), 1.80 (2H, m), 1.65 (2H, m), 1.12 (3H, s), 1.11 (3H, s), 0.94 (9H, t, *J* = 8.0 Hz), 0.66 (6H, q, *J* = 8.0 Hz) ppm; ^{13}C NMR (75 MHz [FIG. 26], $CDCl_3$) δ 139.7, 135.3, 128.3, 128.0,

127.4, 118.6, 104.5, 94.4, 80.0, 78.0, 77.2, 75.7, 73.2, 71.2, 69.2, 58.3, 55.9, 55.8, 51.7, 46.0, 36.3, 29.4, 21.5, 21.1, 7.4 (3C), 5.6 (3C) ppm; HRMS Calcd for $C_{32}H_{56}O_8SiLi$ ($[M + Li]^+$): 603.3904. Found: 603.3900.

[0102] Compound **ent-21**:

[0103] To a solution of olefin **ent-20** (20 mg, 0.033 mmol) and *N*-methylmorpholine-*N*-oxide (12 μL) in 1 mL acetone at 0°C was added OsO_4 (10 μL, 0.1 M solution in *t*–BuOH). The reaction was allowed to warm to RT and stirred overnight. After completion of the reaction, Na_2SO_3 solution was added at 0°C, and stirring was continued for another 1 h. After addition of EtOAc, the organic phase was separated, and the aq. phase was extracted with EtOAc. The combined organic extracts were dried (Na_2SO_4) and concentrated to provide 23 mg of crude intermediate ***iv***, which was used in the next step without further purification. The crude intermediate ***iv*** was dissolved in CH_2Cl_2 and cooled to 0°C. Pyridine (10 μL, 0.126 mmol) and $Pb(OAc)_4$ (20 mg, 0.0432 mmol) were then added, and the mixture was vigorously stirred for 25 min at 0°C. After completion of the reaction, the mixture was filtered through silica gel and washed with ether. The filtrate was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by FC (EtOAc/hexane 1/2) to provide aldehyde intermediate ***v*** (18 mg, 90% for two steps). Intermediate ***v***: $[\alpha]_D^{23} = -54$ (*c* 0.5, $CHCl_3$); IR 2953, 2873, 1733, 1453, 1136, 1106, 1038, 736 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.72 (1H, d, *J* = 1.6 Hz), 7.30 (5H, m), 4.78 (1H, d, *J* = 7.2 Hz), 4.72 (1H, d, *J* = 7.2 Hz), 4.55 (1H, d, *J* = 12.0 Hz), 4.42 (1H, d, *J* = 12.0 Hz), 4.03 (1H, dd, *J* = 1.6, 3.2 Hz), 3.92 (1H, d, *J* = 1.6 Hz), 3.91 (1H, ddd, *J* = 2.8, 2.8, 6.4 Hz), 3.75 (1H, d, *J* = 8.8 Hz), 3.60 (1H, dddd, *J* = 2.8, 6.4, 9.2, 11.6 Hz), 3.47 (1H, ddd, *J* = 2.4, 4.0, 12.0 Hz), 3.43 (3H, s), 3.33 (3H, s), 3.32 (3H, s), 3.31 (1H, d, *J* = 8.8 Hz), 3.29 (3H, s), 1.89

(2H, t, J = 6.4 Hz), 1.68 (1H, dd, J = 11.6, 23.6 Hz), 1.59 (1H, m), 1.13 (3H, s), 1.11 (3H, s), 0.95 (9H, t, J = 8.4 Hz), 0.67 (6H, q, J = 8.4Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 203.1, 139.5, 128.3, 127.5, 127.3, 104.5, 97.5, 82.8, 78.2, 75.8, 73.2, 71.0, 68.4, 58.0, 56.4, 55.9, 51.8, 45.8, 35.8, 31.1, 29.5, 21.5, 21.2, 7.4 (3C), 5.6 3C) ppm. MS (ES) m/z (%) : 621.30 ([M+Na]$^+$, 50). Aldehyde intermediate ***v*** (1.5 g, 2.5 mmol), ^{t}BuOH (43 mL), $NaClO_2$ (849 mg, 7.5 mmol), NaH_2PO_4 (517 mg, 3.75 mmol), 2-methyl-2-butene (43 mL, 2M in THF) and H_2O (8.6 mL) were combined and the mixture was stirred at RT for 1 h. The solvent was removed and the residue was diluted with EtOAc and washed with brine. The organic layers were dried over Na_2SO_4 and concentrated to afford 1.7 g of crude carboxylic acid intermediate ***vi*** which was used in the next step without further purification. To a solution of the crude acid intermediate ***vi*** in ether at 0°C was added a CH_2N_2 solution in ether. After stirring at the same temperature for 1 h, the solvent was removed and the residue was purified was by FC (EtOAc/hexane 1/5) to provide the product methylester **ent-21** (1.095 g, 70% for four steps from **ent-20**). **Ent-21**: $[\alpha]_D^{23}$ = –26 (c 1.0, $CHCl_3$); IR 2951, 1753, 1460, 1206, 1136, 1106, 1038, 735 cm^{-1}; ^{1}H NMR (400 MHz [FIG. 27], $CDCl_3$) δ 7.31 (5H, m), 4.74 (1H, d, J = 7.2 Hz), 4.70 (1H, d, J = 7.2 Hz), 4.55 (1H, d, J = 12.0 Hz), 4.43 (1H, d, J = 12.0 Hz), 4.28 (1H, d, J = 3.2 Hz), 3.96 (1H, d, J = 2.0 Hz), 3.93 (1H, m), 3.78 (1H, d, J = 8.8 Hz), 3.76 (3H, s), 3.62 (1H, m), 3.47 (1H, ddd, J = 2.4, 4.0, 11.6 Hz), 3.40 (3H, s), 3.35(1H, d, J = 8.8 Hz), 3.34 (3H, s), 3.32 (6H, s), 1.89 (2H, t, J = 6.0, 6.4 Hz), 1.69 (1H, dd, J = 12.0, 24.0 Hz), 1.59 (1H, ddd, J = 3.2, 3.2, 12.0 Hz), 1.13 (3H, s), 1.12 (3H, s), 0.95 (9H, t, J = 8.4 Hz), 0.67 (6H, q, J = 8.0 Hz) ppm; ^{13}C NMR (75 MHz [FIG. 28], $CDCl_3$) δ 171.2, 139.4, 128.1, 127.2, 127.1, 104.2, 96.5, 78.2, 76.1, 75.5, 73.0, 71.0, 68.4, 57.8, 56.2, 55.6, 51.9, 51.6, 45.7, 35.7, 29.3, 21.3, 20.9, 7.2 (3C), 5.4 (3C) ppm; HRMS Calcd for $C_{32}H_{56}O_{10}SiLi$ ([M + Li]$^+$): 635.3803. Found: 635.3811.

[0104] Compound **ent-22**:

[0105] To a solution of ester **ent-21** (217mg, 0.345 mmol) in MeOH (22 ml) was added 10 % Pd/C (87 mg). After stirring the reaction mixture under an atmosphere of H_2 (balloon pressure) for 5 h, the reaction mixture was filtered through a plug of Celite, washed with ether, and concentrated to afford 185 mg of product intermediate ***vii*** (98%). $[\alpha]_D^{23} = -50$ (*c* 0.8, $CHCl_3$); IR 3513, 2952, 1751, 1206, 1156, 1106, 1064, 736 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.74 (1H, d, *J* = 7.2 Hz), 4.70 (1H, d, *J* = 6.4 Hz), 4.30 (1H, d, *J* = 3.6 Hz), 3.98 (1H, d, *J* = 2.0 Hz), 3.91 (1H, dd, *J* = 1.6, 9.2 Hz), 3.86 (1H, ddd, *J* = 3.6, 6.4, 6.4 Hz), 3.78 (3H, s), 3.71 (1H, m), 3.52 (1H, ddd, *J* =2.4, 4.8, 11.6 Hz), 3.41 (3H, s), 3.40 (3H, s), 3.39 (3H, s), 3.33 (3H, s), 3.26 (1H, dd, *J* = 7.2, 10.8 Hz), 3.04(1H, m), 1.91 (2H, t, *J* = 6.0, 6.4 Hz), 1.68 (2H, m), 1.10 (3H, s), 1.03 (3H, s), 0.96 (9H, t, *J* = 8.4 Hz), 0.68 (6H, q, *J* = 8.0 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.0, 105.5, 96.5, 78.6, 76.5, 75.8, 70.9, 70.5, 69.6, 57.9, 56.2, 55.6, 51.9, 51.6, 44.9, 36.0, 29.2, 22.3, 21.6, 7.1 (3C), 5.3 (3C) ppm. To a solution of alcohol intermediate ***vii*** (185 mg, 0.3455 mmol) and triethylamine (240 μL, 1.73 mmol) in $DMSO/CH_2Cl_2$ (1/1, 14 mL) was added $Py{\bullet}SO_3$ at 0°C. After 45 min, the reaction mixture was allowed to warm to RT for 75 min, followed by dilution with ether. After washing with water and brine, drying over Na_2SO_4, and concentration, 175 mg of crude aldehyde **ent-22** was obtained and used in the next step without further purification. **Ent-22**: $[\alpha]_D^{23} = -56$ (*c* 1.0, $CHCl_3$); IR 2952, 1753, 1720, 1393, 1210, 1153, 1042, 733 cm^{-1}; ^1H NMR (400 MHz [FIG. 29], $CDCl_3$) δ 4.74 (1H, d, *J* = 7.2 Hz), 4.70 (1H, d, *J* = 6.4 Hz), 4.30 (1H, d, *J* = 3.6 Hz), 3.98 (1H, d, *J* = 2.0 Hz), 3.91 (1H, dd, *J* = 1.6, 9.2 Hz), 3.86 (1H, ddd, *J* = 3.6, 6.4, 6.4 Hz), 3.78 (3H, s), 3.71 (1H, m), 3.52 (1H, ddd, *J* =2.4, 4.8, 11.6 Hz), 3.41 (3H, s), 3.40 (3H, s), 3.39 (3H, s), 3.33 (3H, s), 3.26 (1H, dd, *J* = 7.2, 10.8 Hz),

3.04(1H, m), 1.91 (2H, t, J = 6.0, 6.4 Hz), 1.68 (2H, m), 1.10 (3H, s), 1.03 (3H, s), 0.96 (9H, t, J = 8.4 Hz), 0.68 (6H, q, J = 8.0 Hz) ppm; ^{13}C NMR (75 MHz [FIG. 30], $CDCl_3$) δ 202.4, 171.1, 103.5, 96.5, 78.2, 76.4, 76.2, 69.8, 68.8, 57.9, 56.2, 55.7, 53.0, 52.0, 51.2, 40.9, 35.7, 29.4, 20.1, 17.0, 7.02 (3C), 4.89 (3C) ppm; MS (ES) m/z (%) : 559.36 ([M+Na]$^+$, 20).

[0106] Compound **ent-23**:

[0107] To a solution of diethylmethoxyborane (361 μL, 2.5 mmol) in ether (2.14 mL) was added allylmagnesium bromide (1.0 M in ether, 2.5 mL) drop-wise at 0°C. The white precipitous mixture was stirred at 0°C for 40 min and then allowed to stand for 6 min. A 1.3 mL aliquot of this solution was added drop-wise to a cold (–10°C) solution of aldehyde **ent-22** (175 mg, 0.326 mmol) in 8 mL of ether. After stirring for 25 min at the same temperature, 5 mL aq. sat. NH_4Cl and ether were added. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated. The residue was purified by FC (EtOAc/hexane 5/8) to provide the product **ent-23** (170 mg, 85% for three steps). **Ent-23**: $[\alpha]_D^{23}$ = –52 (c 1.0, $CHCl_3$); IR 3533, 2940, 1750, 1210, 1157, 1107, 1053, 740 cm^{-1}; ^{1}H NMR (400 MHz [FIG. 31], $CDCl_3$) δ 5.95 (1H, dddd, J = 6.8, 6.8, 10.0, 17.2 Hz), 5.06 (2H, m), 4.72 (1H, d, J = 7.2 Hz), 4.68 (1H, d, J = 7.2 Hz), 4.61 (1H, dd, J = 1.6, 10.4 Hz), 4.26 (1H, d, J = 3.6 Hz), 3.96 (1H, d, J = 2.0 Hz), 3.82 (1H, ddd, J = 4.0, 6.4, 6.4 Hz), 3.76 (3H, s), 3.70 (1H, m), 3.66 (1H, s), 3.51 (1H, m), 3.41 (3H, s), 3.39 (3H, s), 3.38 (3H, s), 3.33 (3H, s), 2.17–2.22 (1H, m), 2.04–2.12 (1H, m), 1.98 (1H, dd, J = 3.2, 3.6 Hz), 1.86 (1H, ddd, J = 5.2, 6.0, 14.4 Hz), 1.69 (2H, m), 1.02 (3H, s), 0.95 (3H, s), 0.95 (9H, t, J = 8.0 Hz), 0.68 (6H, q, J = 8.0 Hz) ppm; ^{13}C NMR (75 MHz [FIG. 32], $CDCl_3$) δ 171.0, 137.3, 115.7, 106.3, 96.6, 78.8, 76.8, 75.9, 72.8, 71.4, 70.6, 58.3, 56.4, 55.7, 52.1, 48.3, 36.4, 35.6, 29.2, 21.3,

17.6, 7.3 (3C), 5.5 (3C) ppm. HRMS Calcd for $C_{28}H_{54}O_{10}SiLi$ ($[M + Li]^+$): 585.3646. Found: 585.3658.

[0108] Compound **24**:

[0109] To a solution of alcohol **23** (50 mg, 0.0865 mmol) and 2-naphthaldehyde (67 mg, 0.432 mmol, 5 eq) in CH_2Cl_2 (5 mL) was added TESOTf (10 μL, 0.0433 mmol, 0.5 eq) dropwise at –78°C. After stirring for 15 min at –78°C, 2,6-lutidine (81μl, 0.692 mmol, 8 eq) was added followed by the addition of TESOTf (100 μL, 0.4325 mmol, 5 eq). The reaction mixture was allowed to warm to 0°C over 1 hour, after which no starting alcohol was detected by TLC. Ether (50 mL) and aqueous $NaHCO_3$ (10 mL) were added, the organic phase was separated, and the aqueous phase was extracted with ether (2 × 20 mL). The combined organic solution was dried (Na_2SO_4) and filtered and the solvents were removed under reduced pressure. Flash column chromatography (1:9 EtOAc/Hexanes) provided pure product **24** (47 mg, 81%). $[\alpha]_D^{23}$ = +97 (*c* 0.25, $CHCl_3$); IR 2953, 1750, 1383, 1153, 1130, 1103, 1010, 816, 736 cm^{-1}; ^1H NMR (400 MHz [FIG. 33], $CDCl_3$) δ 7.93 (1H, s), 7.80-7.90 (3H, m), 7.60 (1H, dd, *J* = 1.6, 8.4 Hz), 7.45-7.50 (2H, m), 5.97 (1H, dddd, *J* = 6.8, 6.8, 10.4, 17.6 Hz), 5.86 (1H, s), 5.13 (1H, dd, *J* = 1.6, 17.6 Hz), 5.04 (1H, dd, *J* = 1.2, 10.4 Hz), 4.77 (1H, d, *J* = 6.8 Hz), 4.71 (1H, d, *J* = 6.8 Hz), 4.39 (1H, d, *J* = 3.2 Hz), 4.06 (1H, d, *J* = 2.4 Hz), 4.00-4.08 (2H, m), 3.82-3.90 (1H, m), 3.78 (3H, s), 3.63 (1H, ddd, *J* = 2.4, 5.6, 10.4 Hz), 3.43 (3H, s), 3.39 (3H, s), 3.28 (3H, s), 2.22-2.34 (2H, m), 1.98 (2H, *app*.t, *J* = 6.0 Hz), 1.68-1.78 (2H, m), 1.22 (3H, s), 1.17 (3H, s), 0.99 (9H, t, *J* = 8.0 Hz), 0.70 (6H, q, *J* = 8.0 Hz) ppm; ^{13}C NMR (75 MHz [FIG. 34], $CDCl_3$) δ 171.4, 136.9, 136.3, 133.8, 133.2, 128.5, 128.2, 127.8, 126.4, 126.2, 125.6, 124.2, 116.0, 102.8, 96.7, 94.2, 82.2, 78.5,

76.3, 76.1, 70.8, 66.1, 58.2, 56.4, 55.7, 52.2, 39.4, 35.8, 33.4, 30.2, 20.3, 16.1, 7.4 (3C), 5.7 (3C) ppm. HRMS Calcd for $C_{38}H_{58}O_{10}SiLi$ ($[M+Li]^+$): 709.3959. Found: 709.3943.

[0110] Compound **29:**

[0111] $[\alpha]_D^{23}$ = +55 (*c* 0.15, $CHCl_3$); IR 2928, 1756, 1613, 1467, 1379, 1301, 1258, 1152, 1103, 1052, 834 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ [FIG. 37]) δ 7.94 (1H, s), 7.82-7.90 (3H, m), 7.65 (1H, dd, *J* = 1.2, 8.4 Hz), 7.48-7.53 (2H, m), 6.00 (1H, s), 5.53 (1H, d, *J* = 12.0 Hz), 4.98 (1H, d, *J* = 10.0 Hz), 4.81 (1H, d, *J* = 7.2 Hz), 4.76 (1H, d, *J* = 7.2 Hz), 4.54 (1H, s), 4.31 (1H, dd, *J* = 4.0, 10.0 Hz), 4.24 (1H, d, *J* = 5.2 Hz), 4.08 (1H, d, *J* = 2.4 Hz), 3.76-3.86 (1H, m), 3.60 (1H, ddd, *J* = 2.4, 4.0, 12.0 Hz), 3.50 (3H, s), 3.42 (3H, s), 3.40 (2H, m), 3.37 (3H, s), 3.26 (3H, s), 3.14-3.22 (1H, m), 2.56-2.68 (2H, m), 1.99-2.12 (3H, m), 1.54-1.80 (4H, m), 1.47 (3H, s), 1.21-1.42 (2H, m), 1.26 (3H, s), 1.19 (3H, s), 1.00 (9H, t, *J* = 8.0 Hz), 0.94 (3H, t, *J* = 7.6 Hz), 0.86 (9H, s), 0.73 (6H, q, *J* = 8.0 Hz), 0.00 (3H, s), –0.01 (3H, s) ppm; ^{13}C NMR (75 MHz, $CDCl_3$ [FIG. 41]) δ 169.7, 135.8, 134.1, 134.0, 133.2, 131.2, 128.5, 128.4, 127.9, 126.6, 126.4, 124.2, 102.8, 96.7, 96.1, 82.4, 78.8, 77.9, 77.4, 75.8, 73.7, 70.6, 66.6, 64.9, 58.7, 56.6, 56.5, 55.8, 42.2, 40.4, 39.3, 35.8, 34.5, 30.9, 29.9, 26.2 (3C), 24.8, 19.7, 18.6, 18.1, 15.7, 11.7, 7.4 (3C), 5.7 (3C), –5.2, –5.3 ppm; HRMS Calcd for $C_{52}H_{86}O_{12}Si_2Li$ ($[M+Li]^+$): 965.5818. Found: 965.5820.

[0112] Compound **30:**

[0113] $[\alpha]_D^{23}$ = +50 (*c* 0.2, $CHCl_3$); IR 3453, 2928, 1757, 1460, 1383, 1150, 1102, 1050, 951, 750 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ [FIG. 42]) δ 7.94 (1H, s), 7.82-7.90 (3H, m), 7.64 (1H, dd, *J* = 1.2, 8.8 Hz), 7.50 (2H, m), 6.03 (1H, s), 5.46 (1H, d, *J* = 10.8 Hz), 4.95 (1H, d, *J* = 10.4 Hz), 4.83 (1H, d, *J* = 6.8 Hz), 4.76 (1H, d, *J* = 6.8 Hz), 4.56 (1H, s), 4.33 (1H, dd, *J* = 4.0, 9.6 Hz),

4.28 (1H, d, J = 5.6 Hz), 4.05 (1H, d, J = 2.8 Hz), 3.88 (1H, m), 3.66 (1H, ddd, J = 3.2, 4.8, 11.6 Hz), 3.48 (3H, s), 3.48 (1H, m), 3.43 (3H, s), 3.38 (3H, s), 3.32 (3H, s), 3.29 (1H, m), 3.20 (1H, m), 2.60-2.72 (2H, m), 1.98-2.18 (3H, m), 1.80 (1H, m), 1.50-1.70 (3H, m), 1.56 (3H, s), 1.20-1.44 (2H, m), 1.32 (3H, s), 1.23 (3H, s), 0.87 (3H, t, J = 6.8 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$ [FIG. 43]) δ 169.8, 136.2, 135.6, 134.0, 133.2, 130.8, 128.6, 128.4, 128.0, 126.7, 126.5, 126.4, 124.1, 102.1, 96.7, 96.2, 82.7, 78.7, 77.8, 77.4, 75.2, 73.7, 67.0, 66.4, 64.0, 58.8, 56.6, 56.5, 56.0, 42.6, 40.6, 39.4, 35.7, 33.0, 31.6, 30.5, 24.9, 19.5, 15.8, 11.7 ppm; MS (ES) *m/z* (%): 753.20 ($[M+Na]^+$, 100).

[0114] Compound **ent-31**:

[0115] To a solution of olefin **ent-23** (82 mg, 0.1417 mmol) and *N*-methylmorpholine-*N*-oxide (110 μL) in 4.0 mL acetone at 0°C was added OsO_4 (80 μL, 0.1 M solution in *t*-BuOH). The mixture was allowed to warm to RT and stirred overnight. After completion of the reaction, Na_2SO_3 solution was added at 0°C, and stirring was continued for another 1 h, followed by the addition of EtOAc. The organic phase was separated, and the aq. phase was extracted with EtOAc. The combined organic extracts were dried (Na_2SO_4) and concentrated to provide 92 mg of crude diol intermediate ***viii*** which was used in the next step without further purification. The diol intermediate ***viii*** was dissolved in CH_2Cl_2 and cooled to 0°C. Pyridine (69 μL, 0.85 mmol) and $Pb(OAc)_4$ (132 mg, 0.283 mmol) were then added, and the mixture was vigorously stirred for 1.5 min at 0°C. After completion of the reaction, the mixture was filtered through silica gel and washed with ether. The filtrate was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by FC (EtOAc/hexane 1/2) to give aldehyde **ent-31** (73 mg, 95% for two steps). **Ent-31**: $[\alpha]_D^{23}$ = –30 (*c* 0.8, $CHCl_3$); IR 3510, 2953, 1749, 1461, 1385, 1209, 1155,

1102, 1042, 808, 736 cm^{-1}; ^1H NMR (400 MHz [FIG. 44], $CDCl_3$) δ 9.78 (1H, dd, J = 1.6, 3.2 Hz), 5.17 (1H, dd, J = 4.0, 9.8 Hz), 4.71 (1H, d, J = 6.8 Hz), 4.68 (1H, d, J = 6.8 Hz), 4.28 (1H, d, J = 4.0 Hz), 3.97 (1H, d, J = 2.0 Hz), 3.78 (3H, s), 3.69-3.79 (2H, m), 3.51 (1H, ddd, J = 2.4, 8.4, 8.4 Hz), 3.43 (3H, s), 3.39 (3H, s), 3.34 (3H, s), 2.29-2.34 (2H, m), 1.89-1.94 (2H, m), 1.69-1.73 (2H, m), 1.00 (3H, s), 0.96 (9H, t, J = 8.0 Hz); 0.93 (3H,s), 0.68 (6H, q, J = 8.0 Hz) ppm; ^{13}C NMR (75 MHz [FIG. 45], $CDCl_3$) δ 203.8, 171.1, 106.1, 96.8, 79.4, 76.7, 75.9, 71.6, 71.3, 69.6, 58.4, 56.5, 55.9, 52.3, 52.3, 47.9, 45.3, 36.7, 29.4, 21.4, 17.9, 7.4 (3C), 5.7 (3C) ppm; MS (ES) *m/z* (%): 603.20 ($[M+Na]^+$, 100).

[0116] Compounds **ent-32a** and **ent-32b**:

[0117] To a solution of ketone **ent-6** (90 mg, 0.333 mmol) in ether (1.5 mL) at -78°C was added diisopropylethylamine (83 μL, 0.473 mmol), followed by addition of diethylboron trifluoromethanesulfonate (324 μL, 0.324 mmol). After stirring for 15 min at -78°C and 45 min at -30° C, the opaque white solution was cooled to -78°C followed by the drop-wise addition of a solution of aldehyde **ent-23** in ether (0.5 mL, 0.25 mL rinse). The mixture was stirred for 2 h at this temperature and quenched by the addition of 1.6 mL MeOH. After 5 min, 330 μl pH 7.0 phosphate buffer was added and the mixture was allowed to warm to RT. After a mixture of MeOH and 30% aq. H_2O_2 (2 mL, 2:1) was added and stirring continued for 1 h, the aq. layer was extracted with ether (50 mL × 3). The combined organic layers were washed with aq. sat. $NaHCO_3$ and brine, dried and concentrated. The residue was purified by FC to afford 95 mg of a diastereomeric mixture of aldol products **ent-32a** and **ent-32b** (2:1 ratio as determined by H NMR, yield 87%). **Ent-32a:** $[\alpha]_D^{23} = -40$ (c 0.25, $CHCl_3$); IR 3520, 2954, 1747, 1687, 1460, 1383, 1210, 1101, 1053, 833 cm^{-1}; ^1H NMR (400 MHz [FIG. 46], $CDCl_3$) δ 5.44 (1H, dd, J =

1.6, 10.0 Hz), 4.73 (1H, d, J = 7.2 Hz), 4.69 (1H, d, J = 7.6 Hz), 4.64 (1H, dd, J = 2.0, 10.4 Hz); 4.33 (1H, m), 4.28 (1H, d, J = 3.6 Hz), 3.94 (1H, d, J = 1.6 Hz), 3.83 (1H, ddd, J = 3.6, 6.0, 6.0 Hz), 3.77 (3H, s), 3.70 (1H, m), 3.58 (1H, d, J = 4.4 Hz), 3.54 (1H, dd, J = 5.6, 10.0 Hz), 3.53 (1H, m), 3.50 (1H, dd, J = 5.6, 9.6 Hz), 3.40 (6H, s), 3.38 (3H, s), 3.33 (3H, s), 2.86 (1H, dd, J = 3.2, 17.2 Hz), 2.75 (1H, dd, J = 8.8, 17.6 Hz), 2.71 (1H, m), 1.93 (3H, d, J = 1.2 Hz), 1.85 –2.00 (2H, m), 1.64–1.78 (2H, m), 1.45–1.62 (2H, m), 1.17–1.29 (1H, m), 1.03 (1H, m), 1.02 (3H, s), 0.96 (9H, t, J = 8.4 Hz); 0.94 (3H,s), 0.88 (9H, s), 0.85 (3H, t, J = 8.0 Hz), 0.70 (6H, dq, J = 4.4, 7.2 Hz); 0.03 (3H, s); 0.02 (3H, s); ^{13}C NMR (75 MHz [FIG. 47], $CDCl_3$) δ 207.0, 171.2, 139.8, 137.0, 106.4, 96.8, 79.0, 76.9, 76.1, 71.5, 71.3, 70.6, 66.5, 66.2, 58.3, 56.5, 55.9, 52.3, 49.5, 48.3, 37.4, 36.6, 30.5, 29.5, 26.1 (3C), 24.6, 21.2, 18.5, 17.6, 11.8, 7.5 (3C), 5.6 (3C), –5.18, –5.23 ppm; HRMS Calcd for $C_{42}H_{82}O_{13}Si_2Li$ ($[M+Li]^+$): 857.5454. Found: 857.5452. **Ent-32b**: $[\alpha]_D^{23}$ = –50 (*c* 0.6, $CHCl_3$); IR 3480, 2955, 1750, 1687, 1457, 1383, 1101, 837, 737cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.39 (1H, dd, J = 1.2, 10.4 Hz), 4.82 (1H, d, J = 7.2 Hz), 4.71 (1H, d, J = 7.2 Hz), 4.69 (1H, d, J = 7.2 Hz); 4.34 (1H, m), 4.28 (1H, d, J = 4.0 Hz), 4.22 (1H, s), 3.95 (1H, d, J = 2.0 Hz), 3.78 (3H, s), 3.75 (1H, m), 3.70 (1H, m), 3.47-3.56 (3H, m), 3.40 (3H, s), 3.39 (3H, s), 3.37 (3H, s), 3.33 (3H, s), 3.23 (1H, d, J = 6.0 Hz), 2.82 (1H, dd, J = 6.8, 16.4 Hz), 2.69 (1H, m), 2.56 (1H, dd, J = 5.6, 16.0 Hz), 1.94 (3H, s), 1.87 –1.90 (2H, m), 1.68–1.73 (2H, m), 1.53–1.58 (2H, m), 1.12–1.29 (2H, m), 0.99 (3H, s), 0.97 (9H, t, J = 8.0 Hz); 0.90 (3H,s), 0.87 (9H, s), 0.85 (3H, t, J = 7.2 Hz), 0.70 (6H, q, J = 8.0 Hz); 0.02 (3H, s); 0.01 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 207.7, 171.2, 138.8, 137.5, 106.4, 96.8, 79.4, 75.8, 75.1, 71.5, 71.4, 68.7, 66.3, 58.3, 56.5, 55.9, 52.4, 52.3, 50.2, 48.3, 43.1, 36.7, 36.4, 30.5, 29.4, 26.1 (3C), 24.6, 21.3, 21.1, 18.5, 17.8, 11.8, 7.5 (3C), 5.7 (3C), –5.1, –5.2 ppm.

[0118] Compound **ent-33a**:

[0119] To a solution of the alcohol **ent-32a** (33 mg, 40.7 umol) in CH_2Cl_2 (4 mL) was added 2,6-tert-butyl-methyl-pyridine (256 mg, 1.221mmol) and Me_3OBF_4 (120 mg, 0.814 mmol) at RT. After stirring for 1 h, the mixture was diluted with ether and washed with sat. aq. NH_4Cl and brine. The organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by FC (EtOAc/Hexanes 1/4) to afford 28 mg of product **ent-33a** (85%). **Ent-33a**: $[\alpha]_D^{23} = -45$ (*c* 0.5, CH_2Cl_2); IR 2953.3, 2927, 1757, 1693, 1457, 1257, 1103 cm^{-1}; ^1H NMR (400 MHz [FIG. 48], $CDCl_3$) δ 5.42 (1H, dd, *J* = 1.2, 12.0 Hz), 4.74 (1H, d, *J* = 7.2 Hz), 4.70 (1H, d, *J* = 7.6 Hz), 4.40 (1H, dd, *J* = 4.8, 7.6 Hz); 4.32 (1H, d, *J* = 2.8 Hz), 4.03 (1H, d, *J* = 2.0 Hz), 4.01 (1H, ddd, *J* = 2.1, 5.2, 8.0 Hz), 3.82 (1H, m), 3.79 (1H, m), 3.78 (3H, s), 3.51 (2H, m), 3.40 (3H, s), 3.37 (3H, s), 3.32 (3H, s), 3.30 (3H, s), 2.86 (1H, dd, *J* = 7.2, 16.8 Hz), 2.67 (1H, m), 2.57 (1H, dd, *J* = 5.2, 16.8 Hz), 1.92 (3H, d, *J* = 1.2 Hz), 1.86 –2.02 (2H, m), 1.49–1.76 (4H, m), 1.18–1.26 (2H, m), 1.19 (3H, s), 1.11 (3H, s), 0.95 (9H, t, *J* = 8.0 Hz); 0.94 (3H,s), 0.87 (9H, s), 0.84 (3H, t, *J* = 8.0 Hz), 0.64 (6H, q, *J* = 8.0 Hz); 0.03 (3H, s); 0.02 (3H, s); ^{13}C NMR (75 MHz [FIG. 49], $CDCl_3$) δ 204.0, 171.8, 139.4, 137.3, 108.2, 96.9, 83.9, 78.7, 76.2, 75.7, 74.5, 69.6, 66.3, 65.3, 58.1, 58.0, 56.5, 55.8, 52.1, 47.5, 44.6, 43.2, 36.8, 34.9, 26.1 (3C), 24.6, 21.0, 18.5, 17.9, 11.9, 7.4 (3C), 5.8 (3C), –5.20 (2C) ppm; HRMS Calcd for $C_{42}H_{80}O_{12}Si_2Li$ ($[M+Li-H_2O]^+$): 839.5348. Found: 839.5346.

[0120] Compound **ent-34a**:

[0121] To a solution of ketone **ent-33a** (8 mg) (azeotropically dried with PhH) in 1.5 mL of CH_2Cl_2 was added a 1M solution of (S)-B-Me-CBS reagent in toluene (200 μL, 0.2 mmol) at –30°C followed by the addition of 1 M solution of $BH_3 \cdot Me_2S$ (60 μL, 0.06 mmol) in CH_2Cl_2.

After stirring at –30°C for 1 h, the mixture was allowed to warm to RT and quenched with 1 mL of EtOH and ether. The solution was washed with water and brine, dried (Na_2SO_4) and concentrated. The residue was purified by FC (EtOAc/Hexanes 1/4 to 1/3) to provide pure alcohol intermediate ***ix*** (7.2 mg, 90%). Intermediate ***ix***: $[\alpha]_D^{23} = -65$ (*c* 0.5, $CHCl_3$); IR 3473, 2929, 1753, 1460, 1380, 1257, 1099, 836, 733 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.86 (1H, d, *J* = 10.4 Hz), 4.75 (1H, d, *J* = 7.2 Hz), 4.71 (1H, d, *J* = 7.2 Hz), 4.57 (1H, d, *J* = 6.4 Hz), 4.44 (1H, dd, *J* = 3.6, 8.8 Hz); 4.33 (1H, d, *J* = 2.8 Hz), 4.03 (1H, d, *J* = 1.2 Hz), 4.01 (1H, m), 3.82 (1H, m), 3.78 (3H, s), 3.52 (1H, m), 3.49 (1H, dd, *J* = 5.6, 9.6 Hz), 3.41 (3H, s), 3.38 (3H, s), 3.37 (3H, s), 3.33 (1H, m), 3.31 (3H, s), 3.16 (1H, s), 2.67 (1H, m), 2.00 (1H, ddd, *J* = 5.6, 8.8, 14.0 Hz), 1.90 (1H, ddd, *J* = 4.0, 5.6, 12.8 Hz), 1.81(1H, td, *J* = 3.6, 14.0 Hz), 1.72 (3H, d, *J* = 1.2 Hz), 1.43–1.78 (5H, m), 1.22 (3H, s), 1.13 (3H, s), 1.04–1.24 (2H, m), 0.96 (9H, t, *J* = 7.6 Hz); 0.88 (9H, s), 0.88 (3H, t, *J* = 7.2 Hz), 0.65 (6H, q, *J* = 7.6 Hz); 0.03 (6H, s) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.8, 139.9, 128.8, 108.2, 96.9, 84.2, 78.8, 76.2, 76.0, 75.7, 69.6, 68.8, 67.0, 65.3, 58.1, 56.5, 55.8, 52.2, 44.6, 41.8, 40.8, 36.5, 34.8, 30.5, 29.9, 26.1 (3C), 24.9, 21.0, 20.2, 18.6, 17.9, 12.0, 7.4 (3C), 5.8 (3C), –5.20 (2C) ppm; HRMS Calcd for $C_{42}H_{82}O_{12}Si_2Li$ ($[M+Li-H_2O]^+$): 841.5505. Found: 841.5507. To a stirred solution of alcohol intermediate ***ix*** (2 mg) in 0.5 mL THF at RT was added 0.5 ml of a 0.3 N aq. LiOH solution. The reaction mixture was stirred at RT overnight and diluted with EtOAc, washed with 3 mL of a 1M aq. solution of NaH_2PO_4 and brine. The organic extracts were dried over Na_2SO_4 and concentrated to provide 2 mg of crude carboxylic acid **ent-34a**, which was used in the next step without further purification. **Ent-34a**: IR 3440, 2927, 1732, 1463, 1257, 1104, 837; cm^{-1}; ^1H NMR (400 MHz [FIG. 50], $CDCl_3$) δ 4.84 (1H, d, *J* = 10.0 Hz), 4.79 (1H, d, *J* = 6.8 Hz), 4.71 (1H, d, *J* = 6.8 Hz), 4.53 (1H, dd, *J* = 2.0, 10.0 Hz), 4.47 (1H, dd, *J* = 3.6, 9.2 Hz); 4.30 (1H, d, *J* = 3.6 Hz), 4.04

(1H, m), 4.02 (1H, d, J = 2.0 Hz), 3.80 (2H, m), 3.55 (1H, dd, J = 6.0, 9.6 Hz), 3.49 (1H, m), 3.44 (3H, s), 3.42 (3H, s), 3.37 (3H, s), 3.32 (1H, m), 3.30 (3H, s), 3.27 (1H, m), 2.74 (1H, m), 2.02 (1H, ddd, J = 4.0, 10.0, 14.0 Hz), 1.75–1.90 (4H, m), 1.71 (3H, s), 1.46–1.70 (3H, m), 1.22 (3H, s), 1.10 (3H, s), 1.12–1.28 (2H, m), 0.96 (9H, t, J = 8.0 Hz); 0.88 (9H, s), 0.87 (3H, t, J = 8.0 Hz), 0.64 (6H, q, J = 8.0 Hz); 0.04 (6H, s); ^{13}C NMR (75 MHz [FIG. 51], $CDCl_3$) δ 172.5, 139.9, 128.9, 108.5, 97.1, 84.5, 78.4, 77.0, 76.6, 75.7, 69.4, 69.2, 67.1, 65.2, 58.3, 57.6, 56.5, 55.8, 45.0, 41.6, 41.0, 35.4, 35.3, 30.5, 29.9, 26.1 (3C), 24.9, 20.3, 17.8, 12.1, 7.4 (3C), 5.8 (3C), –5.20 (2C) ppm. MS (ES) *m/z* (%): 843.25 ($[M+Na-H_2O]^+$, 100).

[0122] Compound **36:**

[0123] $[\alpha]_D^{23}$ = +13 (*c* 0.1, $CHCl_3$); IR 2923, 2846, 1634, 1460, 1377, 1263, 1017, 797 cm^{-1}; ^{1}H NMR (400 MHz, C_6D_6 [FIG. 52]) δ 5.74 (1H, dd, J = 3.6, 9.6 Hz), 5.17 (1H, d, J = 7.2 Hz), 5.00 (1H, d, J = 7.2 Hz), 4.81 (1H, d, J = 10.0 Hz), 4.68 (1H, d, J = 1.6 Hz), 4.64 (1H, d, J = 7.2 Hz), 4.53 (1H, td, J = 1.6, 11.2 Hz), 4.18 (1H, d, J = 2.4 Hz), 4.06 (1H, *br.*t, J = 11.8 Hz), 4.05 (1H, dd, J = 3.6, 9.6 Hz), 3.64 (1H, dd, J = 7.6, 9.6 Hz), 3.3.6-3.42 (1H, m), 3.32 (1H, td, J = 2.4, 10.8 Hz), 3.30 (3H, s), 3.16 (3H, s), 3.10 (3H, s), 2.95 (3H, s), 2.83 (1H, *br.*m), 2.15-2.26 (2H, m), 1.89-2.04 (4H, m), 1.84 (1H, *app.*q, J = 11.2 Hz), 1.70 (3H, d, J = 1.2 Hz), 1.53 (1H, dd, J = 4.0, 11.6 Hz), 1.20-1.40 (2H, m), 1.38 (3H, s), 1.27 (3H, s), 1.10 (9H, t, J = 8.4 Hz), 1.02 (9H, s), 0.96 (3H, t, J = 7.6 Hz), 0.82 (6H, *app.*dq, J = 2.8, 8.0 Hz); 0.15 (3H, s), 0.14 (3H, s) ppm; ^{13}C NMR (75 MHz, $CDCl_3$ [FIG. 54]) δ 170.2, 133.5, 130.3, 109.2, 96.5, 83.9, 79.0, 77.8, 77.4, 76.0, 75.6, 72.8, 69.0, 65.5, 65.4, 57.6, 56.9, 56.0, 46.5, 42.5, 41.3, 33.2, 30.5, 30.3, 29.9, 26.2 (3C), 24.7, 18.6, 17.6, 16.8, 12.2, 7.4 (3C), 5.83 (3C), –5.1 (2C) ppm; MS (ES) *m/z* (%): 826.40 ($[M+Na-H_2O]^+$, 60).

[0124] Compound **ent-37**:

[0125] Compound **ent-37** is obtained by stirring compound **ent-36** according to the procedure described below for the preparation of **40**. **Ent-37** (compound formula II of claim 2): $[\alpha]_D^{23}$ = + 16 (*c* 0.08, $CHCl_3$) {**37**: $[\alpha]_D^{23}$ = –20 (*c* 0.05, $CHCl_3$)}; IR 3440, 2924, 1737, 1667, 1460, 1260, 1091, 1017, 801 cm^{-1} ; ^1H NMR (400 MHz [FIG. 55], $CDCl_3$) δ 6.81 (1H, *br.*s), 5.50 (1H, d, *J* = 10.8 Hz), 5.05 (1H, d, *J* = 10.4 Hz), 4.55 (1H, *br.*d, *J* = 8.0 Hz), 4.33 (1H, s), 4.24 (1H, tdd, *J* = 2.4, 4.4, 11.6 Hz), 4.14-4.22 (2H, m), 4.01 (1H, d, *J* = 2.8 Hz), 3.80 (1H, ddd, *J* = 3.2, 4.8, 11.6 Hz), 3.63 (1H, dd, *J* = 3.6, 10.4 Hz), 3.39 (3H, s), 3.32-3.40 (2H, m), 3.34 (3H, s), 3.29 (3H, s), 2.55-2.65 (1H, m), 1.96-2.18 (4H, m), 1.15-1.90 (6H, m), 1.64 (3H, d, *J* = 1.2 Hz), 1.17 (3H, s), 1.09 (3H, s), 0.88 (3H, t, *J* = 7.6 Hz) ppm; MS (ES) *m/z* (%): 571.2 ($[M+Na]^+$, 100).

[0126] Compound **38:**

[0127] $[\alpha]_D^{23}$ = +49 (*c* 0.3, $CHCl_3$); IR 2925, 2853, 1749, 1463, 1377, 1253, 1157, 1106, 1003, 833 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$)= [FIG. 58]) δ 5.80 (1H, dd, *J* = 2.4, 10.0 Hz), 5.06 (1H, d, *J* = 10.0 Hz), 4.95 (1H, d, *J* = 5.2 Hz), 4.81 (1H, d, *J* = 7.2 Hz), 4.72 (1H, d, *J* = 7.2 Hz), 4.43 (1H, d, *J* = 1.2 Hz), 4.32 (1H, *br.*d, *J* = 9.6 Hz), 4.01 (1H, d, *J* = 2.8 Hz), 3.87 (1H, *br.*t, *J* = 11.2 Hz), 3.73 (1H, dd, *J* = 4.0, 10.0 Hz), 3.65 (1H, m), 3.45 (1H, dd, *J* = 4.0, 10.8 Hz), 3.38 (6H, s), 3.32 (3H, s), 3.26 (1H, ddd, *J* = 2.4, 4.8, 10.0 Hz), 3.22 (3H, s), 2.50-2.58 (1H, m), 2.15 (1H, dt, *J* = 3.6, 12.4 Hz), 1.96 (1H, td, *J* = 6.0, 11.6 Hz), 1.92 (1H, ddd, *J* = 2.4, 9.2, 15.2 Hz), 1.83 (1H, ddd, *J* = 4.8, 10.8, 15.2 Hz), 1.77 (1H, dt, *J* = 2.0, 12.4 Hz), 1.70 (3H, d, *J* = 0.4 Hz), 1.58-1.68 (2H, m), 1.41-1.46 (1H, m), 1.25 (3H, s), 1.20-1.30 (2H, m), 1.07 (3H, s); 0.97 (9H, t, *J* = 8.0 Hz), 0.88 (9H, s), 0.82 (3H, t, *J* = 7.6 Hz), 0.66 (6H, q, *J* = 7.8 Hz); 0.03 (3H, s); 0.02 (3H, s) ppm; ^{13}C NMR (75 MHz, $CDCl_3$ [FIG. 59]) δ 171.0, 134.2, 130.2, 109.3, 96.6, 83.8, 78.5, 77.4,

75.2, 74.5, 71.7, 69.2, 65.8, 65.0, 57.9, 57.2, 56.2, 55.9, 47.4, 41.8, 37.7, 34.0, 33.0, 30.5, 29.9, 26.2 (3C), 24.6, 18.6, 18.5, 17.0, 12.1, 7.4 (3C), 5.8 (3C), –5.1, –5.2 ppm; HRMS. Calcd for $C_{41}H_{78}O_{11}Si_2Li$ ($[M + Li]^+$): 809.5242. Found: 809.5238.

[0128] Compound **ent-39**:

[0129] To a solution of PPh_3 (28.4 mg, 0.1073 mmol, azeotropically dried with benzene) in 2 mL of THF was added DIAD (22 μL, 0.1073 mmol) drop-wise at RT. The solution was stirred at RT for 20 min. To this solution was added a solution of crude acid **ent-34a** (7.5 mg, 0.00894 mmol) in 3 mL of THF at 0°C over a period of 2 h via syringe pump. The resulting mixture was stirred for an additional h at this temperature and quenched with water. The aq. layer was extracted with EtOAc and the combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by FC (EtOAc/Hexanes 1/4) to afford 5 mg of product **ent-39** (69%). **Ent-39**: $[\alpha]_D^{23} = -60$ (*c* 0.45, $CHCl_3$); IR 2925, 2853, 1749, 1463, 1377, 1253, 1157, 1106, 1003, 833 cm^{-1}; ^1H NMR (400 MHz [FIG. 60], $CDCl_3$) δ 5.81 (1H, dd, *J* = 2.4, 10.0 Hz), 5.06 (1H, d, *J* = 10.0 Hz), 4.95 (1H, d, *J* = 5.2 Hz), 4.81 (1H, d, *J* = 7.2 Hz), 4.72 (1H, d, *J* = 7.2 Hz), 4.43 (1H, d, *J* = 1.2 Hz), 4.32 (1H, d, *J* = 9.6 Hz), 4.01 (1H, d, *J* = 2.8 Hz), 3.87 (1H, t, *J* = 12.0 Hz), 3.73 (1H, dd, *J* = 4.0, 10.0 Hz), 3.65 (1H, m), 3.45 (1H, dd, *J* = 4.0, 10.8 Hz), 3.38 (6H, s), 3.32 (3H, s), 3.26 (1H, ddd, *J* = 2.4, 4.8,10.0 Hz), 3.22 (3H, s), 2.54 (1H, m), 2.15 (1H, td, *J* = 3.6, 12.8 Hz), 1.96 (1H, td, *J* = 6.0, 10.0 Hz), 1.91 (1H, ddd, *J* = 2.4, 9.2, 15.2 Hz), 1.83 (1H, ddd, *J* = 4.8, 10.8, 15.2 Hz), 1.77 (1H, td, *J* = 2.0, 12.4 Hz), 1.70 (3H, d, *J* = 0.4 Hz), 1.20–1.68 (5H, m), 1.07 (3H, s); 0.97 (9H, t, *J* = 8.0 Hz), 0.88 (12H, s), 0.82 (3H, t, *J* = 7.6 Hz), 0.67 (6H, q, *J* = 7.8 Hz); 0.03 (3H, s); 0.02 (3H, s) ppm; ^{13}C NMR (75 MHz [FIG. 61], $CDCl_3$) δ 171.0, 134.2, 130.2, 109.3, 96.6, 83.8, 78.5, 77.4, 75.2, 74.5, 71.7, 69.2, 65.8,

65.0, 57.9, 57.2, 56.2, 55.9, 47.4, 41.8, 37.7, 34.0, 33.0, 30.5, 29.9, 26.2 (3C), 24.6, 18.6, 18.5, 17.0, 12.1, 7.4 (3C), 5.8 (3C), –5.1, –5.2 ppm; HRMS Calcd for $C_{41}H_{78}O_{11}Si_2Li$ ($[M + Li]^+$): 809.5242. Found: 809.5238.

[0130] Compound **ent-40** [(+)-Peloruside A]:

[0131] To a solution of **ent-39** (2 mg) in CH_3CN/H_2O (9/1, 1 mL) at RT was added a 48% aq. HF solution (58 uL). The reaction mixture was stirred for 27 h and quenched by the addition of sat. aq. $NaHCO_3$ and EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated. FC of the residue (2% to 4% MeOH in CH_2Cl_2) provided compound intermediate ***x*** (i.e. the compound with formula III of claim 3 (1.2 mg, 83%). $[\alpha]_D^{23} = -15$ (*c* 0.4, $CHCl_3$); IR 3435, 2929, 1738, 1455, 1382, 1216, 1154, 1084, 1026, 931 cm^{-1}; ^1H NMR (400 MHz [FIG. 63], $CDCl_3$) δ 6.67 (1H, s), 5.68 (1H, d, *J* = 10.4 Hz), 5.01 (1H, d, *J* = 10.4 Hz), 4.89 (1H, m), 4.81 (1H, d, *J* = 7.2 Hz), 4.71 (1H, d, *J* = 7.2 Hz), 4.63 (1H, s), 4.37 (1H, s), 4.35 (1H, m), 4.25 (1H, ddd, *J* = 2.8, 4.0, 11.2 Hz), 4.04 (1H, d, *J* = 2.8 Hz), 3.98 (1H, br. d, *J* = 8.8 Hz), 3.84 (1H, ddd, *J* = 2.8, 5.2, 11.6 Hz), 3.63 (1H, td, *J* = 2.8, 10.0 Hz), 3.49 (3H, s), 3.41 (3H, s), 3.40 (3H, s), 3.36 (1H, m), 3.34 (3H, s), 3.10 (1H, d, *J* = 7.6 Hz), 2.61 (1H, m), 1.98–2.21 (5H, m), 1.84 (1H, ddd, *J* = 4.4, 11.2, 12.4 Hz), 1.77 (1H, ddd, *J* = 2.0, 4.8, 12.4 Hz), 1.70 (3H, d, *J* = 0.8 Hz), 1.20– 1.60 (3H, m), 1.14 (3H, s), 1.11 (3H, s), 0.87 (3H, t, *J* = 7.6 Hz); ^{13}C NMR (75 MHz [FIG. 64], $CDCl_3$) δ 171.4, 136.9, 130.8, 102.0, 96.9, 78.6, 78.1, 76.3, 74.1, 70.7, 67.3, 67.0, 63.3, 59.3, 56.9, 56.5, 55.9, 44.0, 43.5, 35.9, 34.3, 32.8, 32.0, 29.9, 24.9, 20.9, 17.7, 16.2, 12.4 ppm; MS (ES) *m/z* (%): 615.35 ($[M+Na]^+$, 100). To a solution of intermediate ***x*** (4 mg, 0.00488 mmol) in THF (1.5 mL) was added 4N aq. HCl (1.5 mL). The resulting solution was stirred at RT for 3 h and quenched by the slow addition of sat. aq. $NaHCO_3$. EtOAc was added and the

organic solution was washed with brine and dried over Na_2SO_4. Filtration and concentration followed by FC (4% to 6% MeOH in CH_2Cl_2) provided (+)-Peloruside A (**ent-40**) (1.8 mg, 68%). (+)-Peloruside A can also directly be obtained by stirring compound **ent-39** in a THF/4N aq. HCl mixture as described here for deprotection of intermediate ***x***. **Ent-40 [(+)-Peloruside A]** (compound formula I of claim 1): $[\alpha]_D^{23} = +15.5$ (*c* 0.2, CH_2Cl_2); IR 3447, 2928, 2853, 1729, 1460, 1383, 1273, 1123, 1072, 740 cm^{-1}; ^1H NMR (400 MHz [FIGs 5, 65], $CDCl_3$) δ 6.77 (1H, s), 5.70 (1H, d, *J* = 10.4 Hz), 5.05 (1H, d, *J* = 10.0 Hz), 4.90 (1H, br.d, *J* = 10.4 Hz), 4.54 (1H, d, *J* = 9.6 Hz), 4.44 (1H, s), 4.27 (1H, tdd, *J* = 2.4, 4.4, 11.2 Hz), 4.24 (1H, dd, *J* = 5.2, 10.4 Hz), 4.02 (1H, s), 3.99 (1H, m), 3.82 (1H, ddd, *J* = 2.4, 5.2, 11.2 Hz), 3.65 (1H, ddd, *J* = 4.0, 8.4, 10.4 Hz), 3.48 (3H, s), 3.39 (3H, s), 3.37 (1H, td, *J* = 3.2, 10.4 Hz), 3.31 (3H, s), 3.00 (1H, dd, *J* = 3.2, 8.4 Hz), 2.71 (1H, d, *J* = 9.6 Hz), 2.61 (1H, m), 2.17 (1H, ddd, *J* = 2.0, 10.0, 15.6 Hz), 2.15 (1H, m), 2.07 (1H, ddd, *J* = 4.8, 11.6, 15.6 Hz), 2.03 (1H, ddd, *J* = 4.8, 11.6, 15.6 Hz), 1.81 (1H, m), 1.80 (1H, ddd, *J* = 2.4, 5.2, 12.4 Hz), 1.68 (3H, d, *J* = 0.8 Hz), 1.19–1.60 (4H, m), 1.13 (3H, s), 1.10 (3H, s), 0.87 (3H, t, *J* = 7.2 Hz) ppm; ^{13}C NMR (75 MHz [FIG. 4], $CDCl_3$) δ 174.2, 136.3, 131.4, 102.1, 78.5, 78.1, 76.1, 74.1, 71.1, 70.5, 67.2, 67.1, 63.7, 59.3, 56.3, 55.9, 43.8, 43.5, 35.9, 34.1, 32.8, 31.9, 24.8, 21.0, 17.7, 16.0, 12.5 ppm; HRMS Calcd for $C_{27}H_{48}O_{11}Li$ ($[M + Li]^+$): 555.3357. Found: 555.3338.

[0132] (–)-Peloruside A (**40**):

[0133] To a solution of lactone **38** (4 mg, 0.00488 mmol) in THF (1.5 mL) was added 4N HCl (1.5 mL). The resultant solution was stirred at room temperature for 3 h. Aqueous $NaHCO_3$ was added dropwise followed by extraction with EtOAc. The organic solution was washed with brine and dried over Na_2SO_4. Filtration and concentration followed by flash chromatography

(4%→6% MeOH in CH_2Cl_2) provided (–)-Peloruside **40** (1.8 mg, 65%). $[\alpha]_D^{23} = -16$ (*c* 0.15, CH_2Cl_2); IR 3447, 2928, 2853, 1729, 1460, 1383, 1273, 1123, 1072, 740 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.75 (1H (OH), *br*.s), 5.68 (1H, d, *J* = 10.4 Hz), 5.05 (1H (OH), d, *J* = 10.0 Hz), 4.89 (1H, *br*.d, *J* = 10.4 Hz), 4.53 (1H, d, *J* = 9.6 Hz), 4.43 (1H (OH), s), 4.25 (1H, tdd, *J* = 2.4, 4.4, 11.2 Hz), 4.22 (1H, dd, *J* = 5.2, 10.4 Hz), 4.02 (1H, *br*.s), 3.98-4.02 (1H, m), 3.82 (1H, ddd, *J* = 2.4, 5.2, 11.2 Hz), 3.64 (1H, ddd, *J* = 4.0, 8.0, 10.5 Hz), 3.48 (3H, s), 3.38 (3H, s), 3.36 (1H, dt, *J* = 3.2, 10.5 Hz), 3.31 (3H, s), 3.00 (1H (OH), dd, *J* = 3.2, 8.0 Hz), 2.70 (1H (OH), d, *J* = 9.6 Hz), 2.57-2.66 (1H, m), 2.15 (1H, *br*.dd, *J* = 10.4, 15.5 Hz), 2.12-2.16 (1H, m), 2.07 (1H, ddd, *J* = 4.4, 11.2, 14.6 Hz), 2.02 (1H, *br*.dd, *J* = 11.6, 15.5 Hz), 1.78 (1H, ddd, *J* = 2.4, 5.2, 12.4 Hz), 1.78 (1H, m), 1.67 (3H, d, *J* = 0.8 Hz), 1.53 (1H, q, *J* = 12.0 Hz), 1.40-1.46 (1H, m), 1.40 (1H, *br*.d, *J* = 14.6 Hz), 1.16-1.20 (1H, m), 1.12 (3H, s), 1.10 (3H, s), 0.86 (3H, t, *J* = 7.4 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.0, 136.1, 131.2, 101.9, 78.3, 77.9, 75.9, 73.9, 70.9, 70.3, 67.0, 66.9, 63.5, 59.1, 56.1, 55.7, 43.6, 43.4, 35.7, 33.9, 32.6, 31.7, 24.6, 20.8, 17.5, 15.8, 12.3 ppm; HRMS Calcd for $C_{27}H_{48}O_{11}Li$ ($[M + Li]^+$): 555.3357. Found: 555.3338.

[0134] To a solution of *i*-Pr_2NH (8.07 mL, 57.6 mmol) in 150 ml of THF at 0°C was added *n*-BuLi (21.6 mL, 54 mmol, 2.5 M in hexanes) over 5 min, then the mixture was stirred for another 30 min. After cooling to –78°C, a solution of ketone **14** (8.6 g, 42 mmol) in 15 mL THF was added over 5 min. After stirring for 15 min at –78°C, the solution was allowed to warm to –40°C over a period of 1h. The reaction mixture was cooled to –78°C, and a solution of aldehyde **70** (8.3 g, 36 mmol) in THF (10 mL) was added dropwise over 15 min. The resulting mixture was stirred for 45 min at –78°C and quenched by the addition of AcOH in ether (2 mL), and aq. saturated $NaHCO_3$. The aqueous layer was extracted with Et_2O, and the combined organic layers

were washed with water and brine. The organic extracts were dried over $MgSO_4$ and concentrated and the residue was purified by FC (EtOAc/Hexane, 1:4) to afford 14 g (89 %) of product.

[0135] To a solution of aldol product (13 g, 29.9 mmol), DMSO (50 mL) and Et_3N (10 mL, 71.6 mmol) in 50 mL CH_2Cl_2 at 0°C was added Py•SO_3 (11.0 g, 69.0 mmol). After stirring at this temperature for one hour, a saturated aqueous NH_4Cl solution (300 mL) was added and the mixture was extracted with ether (150 mL × 3). The organic layers were combined and washed with water and brine and dried over $MgSO_4$. Concentration provided a residue that was purified by FC (5% to 15% EtOAc in hexanes) to afford 10 g (77.4%) of diketone **71**. **71**: $[\alpha]_D^{23} = +41.0$ (*c* 0.4, $CHCl_3$); IR 2929, 2857, 1603, 1472, 1255, 1095, 836, 776 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.30 (5H, m), 5.88 (1H, dddd, *J* = 6.8, 6.8, 10.4, 18.0 Hz), 5.66 (1H, s), 5.07 (2H, m), 4.51 (2H, s), 4.20 (1H, m), 3.45 (1H, d, *J* = 8.8 Hz), 3.42 (1H, d, *J* = 8.8 Hz), 2.43 (1H, dd, *J* = 4.8, 13.6 Hz), 2.36 (1H, dd, *J* = 8.0, 13.6 Hz), 2.27(2H, m), 1.17 (3H, s), 1.16 (3H, s), 0.85 (9H, s), 0.04 (3H, s), –0.02 (3H, s) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.4, 191.9, 138.6, 134.4, 128.5 (2C), 127.6 (3C), 117.9, 98.7, 76.8, 73.5, 69.6, 46.2, 44.2, 42.6, 26.0, 22.9 (3C), 22.8, 18.2, –4.42, –4.83 ppm. MS (ES) *m/z* (%) : 455.20 ($[M+Na]^+$, 100).

[0136] To a solution of diketone **71** (9 g, 21 mmol) in 100 mL of MeOH was added *p*-TsOH (600 mg, 3.15 mmol) at 0°C. After stirring at this temperature for 0.5 h, the ice-bath was removed and the mixture was allowed to warm to RT and stirred for 4 h. The solvent was removed by vacuum and the residue was purified by FC (EtOAc/Hexane, 1:6 to 1:4) to provide the product **72** in 95% yield. **72**: $[\alpha]_D^{23} = -10.0$ (*c* 1.0, $CHCl_3$); IR 2973, 2872, 1668, 1599, 1454, 1336, 1242, 1095, 922, 739, 699 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.27-7.36 (5H, m),

5.77 (1H, dddd, J = 6.8, 6.8, 10.4, 16.0 Hz), 5.47 (1H, s), 5.14 (1H, d, J = 16.0 Hz), 5.13 (1H, d, J = 10.4 Hz), 4.49 (2H, s), 4.32 (1h, ddd, J = 6.0, 6.0, 17.2 Hz), 3.40 (1H, d, J = 8.8 Hz), 3.35 (1H, d, J = 8.8 Hz), 2.43 (4H, m), 1.16 (3H, s), 1.15 (3H, s) ppm; ^{13}C NMR (75MHz, $CDCl_3$): 193.4, 181.4, 138.4, 132.5, 128.4, 127.6, 127.5, 118.6, 103.1, 78.4, 76.5, 73.3, 41.4, 40.5, 38.6, 23.1, 23.0 ppm.

[0137] To a solution of dihydropyranone **72** (0.05 mmol/mL) in MeOH at RT was added $CeCl_3 \cdot H_2O$ (1.0 eq). The mixture was cooled to –30°C, followed by addition of $NaBH_4$ (1.5 eq). After 10 min, another portion $NaBH_4$ (0.5 eq) was added. The mixture was stirred at –30°C for 30 min, followed by quenching with sat. aq. $NaHCO_3$ (3 mL) and brine (20 mL). The aqueous phase was extracted with Et_2O (30 mL × 3), and the combined organic extracts were dried over $MgSO_4$. Concentration provided a residue that was purified by FC (EtOAc/Hexane, 1:4) to provide glycal **73** in 90% yield. **73**: $[\alpha]_D^{23}$ = –12.0 (c 0.9, $CHCl_3$); IR 3387, 2927, 2860, 1663, 1457, 1277, 1100, 1073, 1030, 917, 733, 700 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26-7.34 (5H, m), 5.80 (1H, dddd, J = 6.8, 6.8, 10.0, 16.8 Hz), 5.10 (1H, d, J = 16.8 Hz), 5.00 (1H, d, J = 10.0 Hz), 4.67 (1H, dd, J = 2.0, 2.0 Hz), 4.51 (2H, s), 4.45 (1H, dddd, J = 2.0, 7.0, 9.2, 9.2 Hz), 3.90 (1H, dddd, J = 2.0, 7.0, 7.0, 12.0 Hz), 3.35 (1H, d, J = 8.8 Hz), 3.28 (1H, d, J = 8.8 Hz), 2.36 (1H, ddd, J = 6.8, 14.0, 14.0 Hz), 2.30 (1H, ddd, J = 6.8, 14.0, 14.0 Hz), 2.11 (1H, dddd, J = 16.8, 6.8, 1.6, 1.6 Hz), 1.48 (1H, ddd, J = 13.2, 11.6, 9.2 Hz), 1.35 (1H, d, J = 7.2Hz), 1.08 (3H, s), 1.07 (3H, s) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.4, 139.0, 134.3, 128.4, 127.6, 127.5, 117.4, 99.5, 76.9, 74.3, 73.3, 64.2, 39.8, 39.6, 37.4, 23.4 ppm.

[0138] To a solution of glycal **73** (790 mg, 2.61 mmol) in CH_2Cl_2 (45 mL) and MeOH (15 mL) at 0°C was added $NaHCO_3$, followed by *m*-CPBA (541 mg, 3.135 mmol) in two portions over 10

min. The resultant suspension was stirred at 0°C for 40 min, followed by the addition of Et_3N (6 mL). After stirring for 15 min, the mixture was poured into hexanes (100 mL), and passed through a pad of celite (Et_2O rince). After concentration of the filtrate, the residue was purified by FC (EtOAc/Hexane/Et_3N, 30:70:0.2) to give 690 mg product **74** (57.4%) and 30 mg of a diastereoisomer (3.3%). **74**: $[\alpha]_D^{23} = -20.0$ (*c* 0.8, $CHCl_3$); IR 3412, 2925, 1642, 1454, 1380, 1064, 919, 737, 698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.33 (5H, m), 5.87 (1H, dddd, *J* = 6.8, 6.8, 10.8, 16.4 Hz), 5.10 (1H, d, *J* = 16.4 Hz), 5.05 (1H, d, *J* = 10.8 Hz), 4.55 (1H, d, *J* = 11.6 Hz), 4.41 (1H, d, *J* = 11.6 Hz), 3.90 (1H, dddd, *J* = 4.0, 4.0, 11.0, 11.0 Hz), 3.72 (1H, dd, *J* = 4.0, 4.0 Hz), 3.58 (1H, dddd, *J* = 3.2, 5.6, 8.0, 12.0 Hz), 3.44 (1H, d, *J* = 9.2 Hz), 3.37 (1H, d, *J* = 9.2 Hz), 3.31 (3H, s), 2.55 (1H, d, *J* = 11.0 Hz), 2.29 (1H, ddd, *J* = 6.8, 13.6, 13.6 Hz), 2.25 (1H, ddd, *J* = 6.8, 13.6, 13.6 Hz), 1.65 (1H, ddd, *J* = 3.0, 4.2, 12.0 Hz), 1.55 (1H, dd, *J* = 12.0, 24.0 Hz), 1.11 (3H, s), 1.03 (3H, s) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.3, 134.6, 128.7, 128.1, 127.9, 117.2, 103.5, 77.1, 73.8, 70.9, 69.5, 67.1, 52.0, 45.5, 40.3, 33.5, 22.8, 22.2 ppm.

[0139] To a solution of diol **74** (5.55 g, 15.8 mmol) in dry THF at 0°C was added KO^{t}Bu (3.0 g, 25.4 mmol). After stirring for 15 min, CH_3I (23 mL, 369 mmol) was added dropwise and the reaction mixture was stirred for 30 min at 0°C, followed by quenching with saturated aqueous NH_4Cl. The phases were separated and the aqueous layer was extracted with ether. The combined organic phases were dried and concentrated in vacuo to afford 5.73 g crude product which was used in the next step without further purification.

[0140] To a solution of alcohol (5.73 g, 15.7 mmol) in CH_2Cl_2 (60 mL) at –78°C was added 2,6-lutidine (5 mL, 42.5 mmol) and TESOTf (6.4 mL, 27.8 mmol). After 2 h, the reaction mixture was diluted with Et_2O (300 mL) and NH_4Cl (100 mL). The organic layer was washed with 100

mL saturated aq. NH_4Cl, and dried over $MgSO_4$. Concentration provided a residue that was purified by FC (1% EtOAc in hexanes) to give product **75** (4.5 g, 81% for two steps). **75**: $[\alpha]_D^{23}$ = –11.0 (*c* 0.8, $CHCl_3$); IR 2953, 2876, 1454, 1380, 1139, 1098, 1040, 1004, 734, 696 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.30 (5H, m), 5.88 (1H, dddd, *J* = 6.8, 6.8, 10.0, 16.8 Hz), 5.05 (2H, m), 4.52 (1H, d, *J* = 12 Hz), 4.44 (1H, d, *J* = 12), 3.93 (1H, d, *J* = 2 Hz), 3.79 (1H, d, *J* = 8.8 Hz), 3.54 (1H, m), 3.47 (1H, m), 3.32 (3H, s), 3,31 (3H, s), 2.28 (2H, m), 1.59 (2H, m), 1.11 (6H, s), 0.94 (9H, t, *J* = 8.4 Hz), 0.63 (6H, q, *J* = 8.4 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.8, 134.8, 128.3, 127.2, 117.1, 104.4, 77.1, 75.8, 73.3, 71.5, 71.2, 55.8, 51.7, 46.1, 40.5, 28.8, 21.5, 21.0, 7.4 (3C), 5.6 (3C) ppm; HRMS Calcd for $C_{27}H_{46}O_5SiLi$ ($[M + Li]^+$): 485.3275. Found: 485.3262.

[0141] To a solution of olefin **75** (1 g, 2.1 mmol) and 4-methylmorpholine *N*-oxide (520 μL, 2.5 mmol) in acetone at 0°C was added OsO_4 (420 μL, 0.1 M solution in *t*-BuOH). The mixture was stirred for 16 h at RT, followed by the addition of aq. Na_2SO_3 at 0°C. After 1 h, EtOAc was added and the aqueous phase was extracted with EtOAc. The combined organic extracts were dried (Na_2SO_4) concentrated to provide 1.05 g crude diol.

[0142] The diol was dissolved in CH_2Cl_2 and cooled to 0°C. Pyridine (639 μL, 7.84 mmol) and $Pb(OAc)_4$ (1.26 g, 2.24 mmol) were added portion-wise over 5 min, and the mixture was vigorously stirred for 25 min at 0°C. After completion of the reaction, the mixture was filtered through silica gel and washed with ether. The organic extract was washed with brine and dried over Na_2SO_4. Concentration provided a residue that was purified by FC (EtOAc/hexane, 1:8) to give the aldehyde **76** (850 mg, 85% for two steps). **76**: $[\alpha]_D^{23}$ = –20.0 (*c* 0.75, $CHCl_3$); IR 2954, 2877, 1455, 1384, 1140, 1101, 1039, 735 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.85 (1H, t, *J* =

2.0 Hz), 7.32 (5H, m), 4.47 (1H, dd, J = 12.4 Hz), 4.08 (1H, dddd, J = 4.4, 4.4, 7.2, 12.0 Hz), 3.92 (1H, d, J = 3.6 Hz), 3.73 (1H, d, J = 8.8 Hz), 3.38 (3H, s), 3.32 (3H, s), 3.53 (1H, m), 3.31 (1H, d, J = 8.8 Hz), 2.68 (1H, ddd, J = 2.4, 7.6, 16.8 Hz), 2.54 (1H, ddd, J = 2.0. 4.4, 16.8 Hz), 1.67 (2H, m), 1.10 (3H, s), 1.09 (3H, s), 0.94 (9H, t, J = 8.0 Hz), 0.65 (6H, q, J = 8.0 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.2, 139.6, 128.4, 127.4, 127.3, 104.6, 76.6, 75.7, 73.3, 70.9, 67.5, 56.0, 52.0, 49.5, 45.8, 29.1, 21.5, 21.2, 7.3 (3C), 5.6 (3C) ppm; MS (ES) m/z (%) : 503.23 ($[M+Na]^+$, 20).

[0143] To a solution of 1-(methoxymethyl)oxy-2-propene (7.05 mmol, 1.3 eq) in 45 mL of THF at –78°C was added *sec*-BuLi (5.0 mL, 1.3 M solution in cyclohexane, 6.5 mmol, 1.2 eq) over 15 min. The resulting yellow solution was maintained for 30 min at the same temperature, followed by the addition of a solution of (–)-B-methoxydissopinocampheylborane (2.226 g, 7.05 mmol) in THF (6 mL) over 15 min (the yellow solution became colorless). After stirring for 40 min at –78°C, and 2 h at –10°C, the solution was cooled back to –100°C followed by the drop-wise addition (20 min) of a solution of aldehyde **76** (2.6 g, 5.42 mmol) in THF (1 M). After stirring at –100°C for 3 h, the mixture was allowed to slowly reach RT. The solvent was removed in vacuo and the residue dissolved in ether, followed by aq. NaOH (2 M, 6 mL) and 30% aq. H_2O_2 (1.6 mL). After stirring for 16 h at RT, ether was added and the organic extract was washed with brine and dried over Na_2SO_4. Concentration provided a residue that was purified by FC (EtOAc/hexane, 1:10) to provide the product **77** (2.05 g) and a diastereoisomer (525 mg) total yield is 81%. **77**: $[\alpha]_D^{23}$ = –30.0 (c 1.0, $CHCl_3$); IR 3426, 2927, 1456, 1136, 1098, 1038, 733 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (5H, m), 5.82 (1H, ddd, J = 6.8, 10.4, 17.2 Hz), 5.32 (2H, m,), 4.73 (1H, d, J = 6.4 Hz), 4.62 (1H, d, J = 6.4 Hz), 4.52 (1H, d, J = 12.8 Hz), 4.44 (1H, d, J =

12.8 Hz), 4.07 (1H, s), 4.03 (1H, t, J = 5.6, 6.8 Hz), 3.89 (1H, d, J = 2.4 Hz), 3.88 (1H, m), 3.78 (1H, m), 3.49 (1H, d, J = 9.2 Hz), 3.48 (1H, m), 3.39 (3H, s), 3,38 (3H, s), 3.31 (3H, s), 3.29 (1H, d, J = 9.2 Hz), 1.75 (2H, m), 1.64 (2H, m), 1.11 (6H, s), 0.92 (9H, t, J = 8.4 Hz), 0.63 (6H, q, J = 8.4 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.0, 135.0, 128.3, 128.0, 127.5, 119.0, 105.0, 94.4, 80.4, 76.7, 73.3, 73.0, 71.5, 55.8, 55.7, 51.7, 45.1, 38.6, 29.7, 22.0, 21.7, 7.4 (3C), 5.6 (3C). HRMS Calcd for $C_{31}H_{54}O_8SiLi$ ($[M + Li]^+$): 589.3748. Found: 589.3765. **Isomer**: IR 3473, 2940, 2866, 1150, 1093, 1030, 733 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (5H, m), 5.67 (1H, ddd, J = 8.0, 10.8, 17.2 Hz), 5.32 (2H, m), 4.75 (1H, d, J = 6.8 Hz), 4.59 (1H, d, J = 6.8 Hz), 4.53 (1H, d, J = 12.0 Hz), 4.43 (1H, d, J = 12.0 Hz), 3.93 (1H, m), 3.91 (1H, d, J = 2.4 Hz), 3.89 (1H, m), 3.85 (1H, d, J = 7.2 Hz), 3.72 (1H, d, J = 8.8 Hz), 3.50 (1H, ddd, J = 2.4, 5.2, 11.6 Hz), 3.40 (3H, s), 3.33 (1H, d, J = 8.8 Hz), 3.31 (3H, s), 2.75 (1H, d, J = 3.2 Hz), 1.52–1.71 (4H, m), 1.10 (3H, s), 1.09 (3H, s), 0.93 (9H, t, J = 8.0 Hz), 0.65 (6H, q, J = 8.0 Hz).

[0144] To a solution of alcohol **77** (45 mg, 0.077 mmol) in dry THF at 0°C was added KOBu^t (16 mg, 0.135 mmol). After stirring for 15 min, CH_3I (30 μL, 0.477mmol) was added drop-wise and the reaction mixture was stirred for 30 min at 0°C and quenched with saturated aqueous NH_4Cl. The phases were separated and the aqueous layer was extracted with ether. The combined organic extracts were dried and concentrated in vacuo. The residue was purified by FC (EtOAc/hexane, 1:5) to provide the product **ent-20** (42 mg, 91%). **Ent-20**: $[\alpha]_D^{23}$ = –24.0 (c 0.5, $CHCl_3$); IR 2947, 1459, 1099, 1039, 740 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (5H, m), 5.83 (1H, dddd, J = 7.2, 7.6, 10.0, 17.2 Hz), 5.26 (2H, m), 4.70 (1H, d, J = 6.4 Hz), 4.58 (1H, d, J = 6.4 Hz), 4.53 (1H, d, J = 12.4 Hz), 4.42 (1H, d, J = 12.4 Hz), 4.16 (1H. dd, J = 4.4, 7.2 Hz), 3.93 (1H, d, J = 1.6 Hz), 3.78 (1H, d, J = 8.8 Hz), 3.67 (1H, m), 3.47 (1H, m), 3.38 (3H, s), 3.37 (3H,

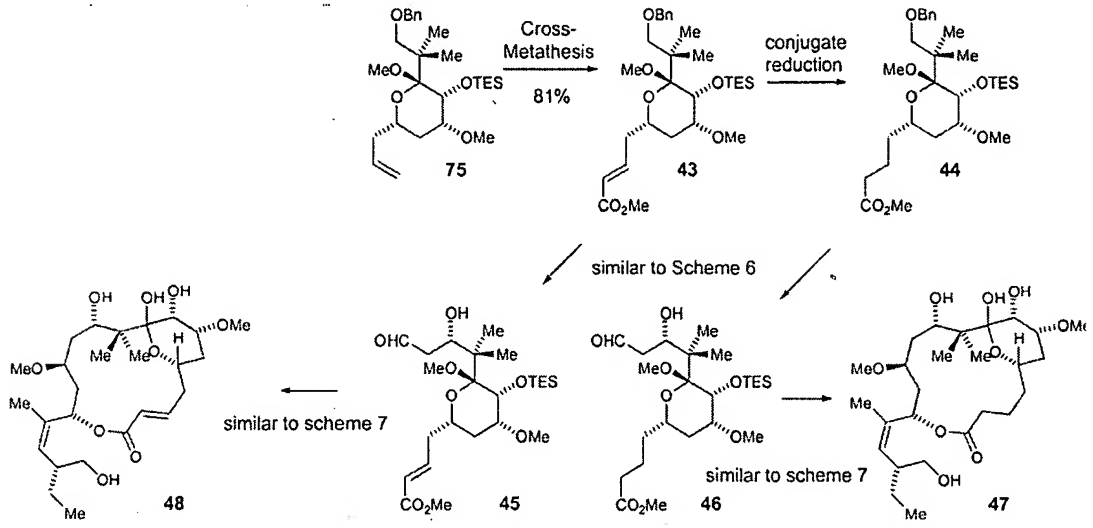
s), 3.34 (1H, d, J = 8.8 Hz), 3.33 (3H, s), 3.32 (3H, s), 1.80 (2H, m), 1.65 (2H, m), 1.12 (3H, s), 1.11 (3H, s), 0.94 (9H, t, J = 8.0 Hz), 0.66 (6H, q, J = 8.0 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.7, 135.3, 128.3, 128.0, 127.4, 118.6, 104.5, 94.4, 80.0, 78.0, 77.2, 75.7, 73.2, 71.2, 69.2, 58.3, 55.9, 55.8, 51.7, 46.0, 36.3, 29.4, 21.5, 21.1, 7.4 (3C), 5.6 (3C) ppm; HRMS Calcd for $C_{32}H_{56}O_8SiLi$ ($[M + Li]^+$): 603.3904. Found: 603.3900.

[0145] To a solution of olefin **75** (400 mg, 0.83551mmol) in CH_2Cl_2 (4 mL) at RT was added methyl acrylate (304 μL, 3.34 mmol), followed by the addition of Grubbs's second generation Ru-alkylidene catalyst (71 mg, 0.0836 mmol). The mixture was kept at 38°C for 15 h, and another portion (71 mg) of catalystwas added. After stirring for 6 h, the solvent was removed and the residue was purified by FC (EtOAc/hexane, 1:10) to give the product **43** (364 mg, 81%). **43**: IR 2952, 1731, 1660, 1455, 1435, 1328, 1270, 1038, 805, 736 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (4H, m), 7.26 (1H, m), 7.02 (1H, ddd, J = 7.2, 7.6, 16.0 Hz), 5.91 (1H, d, J = 16.0 Hz), 4.52 (1H, d, J = 12.0 Hz), 4.45 (1H, d, J = 12.0 Hz), 3.95 (1H, d, J = 2.0 Hz), 3.77 (1H, d, J = 8.8 Hz), 3.73 (3H, s), 3.66 (1H, m), 3.48 (1H, ddd, J = 2.0, 4.4, 11.2 Hz), 3.32 (3H, s), 3.31 (3H, s), 2.44 (2H, m), 1.61 (2H, m), 1.11 (3H, s), 1.10 (3H, s), 0.93 (9H, t, J = 8.0 Hz), 0.65 (6H, q, J = 8.0 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.9, 145.2, 139.7, 128.3 (2C), 127.4 (2C), 127.3, 123.5, 104.5, 76.8, 75.7, 73.2, 70.9, 70.5, 55.9, 51.8, 51.6, 46.0, 38.7, 28.9, 21.5, 21.1, 7.3 (3C), 5.5 (3C) ppm.

Example 4: Synthesis of C2,C3 Peloruside Analogs

[0146] Scheme 9 illustrates a strategy for producing peloruside analogs that lack C2,C3 hydroxy functionality. Olefin cross-metathesis of olefin **76** with methyl acrylate produces compound **43** in 81% yield. Conjugate reduction of this material provides the saturated equivalent **44**. Both of

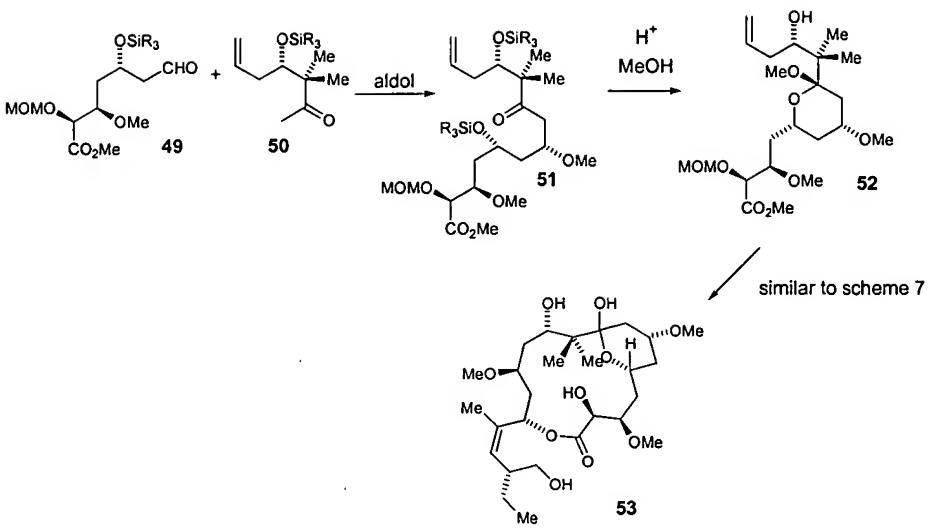
these materials can be elaborated into peloruside analogs **47** and **48**, according to similar procedures as outlined in schemes 6 and 7.



Scheme 9

Example 5: Synthesis of Peloruside analogs that lack C8 hydroxy functionality

[0147] For producing Peloruside analogs that lack the C8 hydroxyl group, compound **49** is reacted with compound **50** via an aldol reaction (see scheme 10 below). Treatment of the resulting aldol product **51** under acidic conditions in methanol solvent affords tetrahydropyranyl derivative **52**. This compound is elaborated into Peloruside analog **53**, according to similar procedures as outlined in scheme 7.



Scheme 10

Example 6: Cytotoxic Effect of Peloruside A on Various Cancer Cell Lines

[0148] The object of the present study is to investigate the effect of a compound of the present invention, Peloruside A, on the proliferation of various tumor cell lines, listed in Table 1. The effects of Peloruside A were compared to the effects of microtubule-stabilizing drug, Taxol®, on cell proliferation. The taxanes, such as paclitaxel or Taxol®, belong to a class of anticancer drugs that stabilize microtubules and regulate tumor cell death. Synthetic (+)-Peloruside A was used for the present example.

Table 1: Cell Lines Used in Cytotoxicity Assay

Cell Lines	Cancer Type	Cells/Well
MDA-MB-231	Breast	125
LoVo	Colon	250
NCI-H1395	Lung	2000
NCI-H2887	Lung	500
NCI-H460	Lung	100

Table 1: Cell Lines Used in Cytotoxicity Assay

Cell Lines	Cancer Type	Cells/Well
NCI-H23	Lung	250
DU-145	Prostate	200
BT-549	Breast	250
Capan-1	Pancreas	1000
PC-3	Prostate	250
Hep-G2	Liver	500
SK-HEP-1	Liver	250
HCT-116	Colon	125
HCT-15	Colon	125
SK-MEL-28	Melanoma	250
SK-MEL-5	Melanoma	1000
Mia PaCa-2	Pancreas	250
SK-OV-3	Ovarian	250
CAKI-1	Renal	500
A498	Renal	125

[0149] Cells were plated on Day 0 in 180 μL of media (RPMI-1640, + 10% FBS, + gentamycin) in a 96-well plate as indicated in Table 1. Cells were treated on Days 1, 3, 5, and 7 with 5 μL of media, vehicle, or drug. Peloruside A was resuspended in 0.1% DMSO and added to wells to a final concentration of 0.01, 0.1, 1, 10 or 100 nM. Taxol® (Sigma-Aldrich) was resuspended in 0.1% ethanol and added to cells to a final concentration of 0.1, 1, 10, 100, or 1000 nM.

[0150] The amount of surviving cells was measured using the sulforhodamine B (SRB) assay as previously described (Skehan et al., *New colorimetric cytotoxicity assay for anticancer-drug screening*. J. Natl. Cancer Inst., 1990. 82(13): p. 1107-12). Cells were fixed on Days 5, 7, and 9 (representing 4, 6, and 8 days of exposure to drug or control, respectively) with 50% (w/v) trichloroacetic acid for 1 hour at 4°C, rinsed thoroughly with water, and dried overnight. The

dried cells were stained with 4% sulforhodamine B in 1% acetic acid, rinsed thoroughly and dried overnight. The bound dye was resuspended in 10 mM Tris and the absorbance was read at 492 nm. Percent growth inhibition (GI) was calculated as follows: %GI = (1 – (absorbance sample/absorbance vehicle)) x 100. The absorbance value for each drug concentration and control represents the average of 6 replicates. The %GI was plotted against the log[drug] to generate the growth curves. The concentration of drug required to inhibit growth by 50% (GI_{50}) was calculated for each time point. The results of the assays are shown in Figures 66-73 and Table 2.

[0151] Peloruside A inhibited proliferation of a panel of tumor cell lines, including cells derived from colon, pancreas, melanoma, ovarian, renal, liver lung, breast and prostate tissue. Peloruside A treatment created a cytotoxic effect similar to Taxol® treatment of the same cells. In fact, Peloruside A is more effective at regulating cell proliferation in HCT-15 cells in comparison to Taxol® (GI_{50} of 14.32 nM for Peloruside A in comparison to 49.1 nM for Taxol® after 4 days of treatment with drug). A similar effect was observed with A498 cells (GI_{50} of 7.3 nM for Peloruside A in comparison to 46.1 nM for Taxol® after 4 days of treatment with drug). These studies suggest that the Peloruside A functions through disruption of microtubule dynamics in a manner similar to Taxol®.

Table 2: GI_{50} of Peloruside A and Taxol® on Cancer Cell Lines

Cell Line	Type of Cancer	Paclitaxel G150	Peloruside G150
LoVo	Colon	4 Days: 1.91 nM 6 Days: 1.95 nM 8 Days: 3.92 nM	4 Days: 11.77 nM 6 Days: 7.34 nM 8 Days: 6.14 nM
HCT-15*	Colon	4 Days: 49.1 nM 6 Days: 24.0 nM 8 Days: 26.7 nM	4 Days: 14.32 nM 6 Days: 9.23 nM 8 Days: 11.7 nM
HCT-116	Colon	4 Days: 1.0 nM 6 Days: 0.58 nM 8 Days: 0.30 nM	4 Days: 3.64 nM 6 Days: 5.81 nM 8 Days: 1.79 nM
Capan-1	Pancreas	4 Days: 5.68 nM 6 Days: 0.91 nM 8 Days: 1.43 nM	4 Days: 29.77 nM 6 Days: 6.8 nM 8 Days: 5.21 nM
Mia PaCa-2	Pancreas	4 Days: 0.73 nM 6 Days: 0.53 nM 8 Days: 0.27 nM	4 Days: 9.65 nM 6 Days: 1.76 nM 8 Days: 2.44 nM
SK-MEL-5	Melanoma	4 Days: 0.82 nM 6 Days: 1.0 nM 8 Days: 0.27 nM	4 Days: 9.42 nM 6 Days: 1.76 nM 8 Days: 2.44 nM
SK-MEL-28	Melanoma	4 Days: 0.76 nM 6 Days: 0.20 nM 8 Days: 0.01 nM	4 Days: 23.3 nM 6 Days: 7.29 nM 8 Days: 2.07 nM
SK-OV-3	Ovarian	4 Days: ND 6 Days: 2.21 nM 8 Days: 1.07 nM	4 Days: 8.81 nM 6 Days: 8.51 nM 8 Days: 5.77 nM
CAKl-1	Renal	4 Days: 4.52 nM 6 Days: 1.21 nM 8 Days: 1.17 nM	4 Days: 12.5 nM 6 Days: 3.97 nM 8 Days: 3.00 nM
A498	Renal	4 Days: 46.1 nM 6 Days: 9.75 nM 8 Days: 7.57 nM	4 Days: 7.3 nM 6 Days: 1.9 nM 8 Days: 2.75 nM
PC-3	Prostate	4 Days: 0.78 nM	4 Days: 11.63 nM

*Peloruside A is more effective at inhibiting cell proliferation then Paclitaxel in HTC-15 cells.

Table 2: GI_{50} of Peloruside A and Taxol® on Cancer Cell Lines

Cell Line	Type of Cancer	Paclitaxel G150	Peloruside G150
		6 Days: 0.47 nM 8 Days: 0.27 nM	6 Days: 6.50 nM 8 Days: 2.46 nM
DU-145	Prostate	4 Days: 0.54 nM 6 Days: 0.53 nM 8 Days: 0.49 nM	4 Days: 9.20 nM 6 Days: 3.25 nM 8 Days: 2.94 nM
BT-549	Breast	4 Days: 1.76 nM 6 Days: 1.2 nM 8 Days: 0.18 nM	4 Days: 38.76 nM 6 Days: 4.28 nM 8 Days: 5.73 nM
MB-MDA-231	Breast	4 Days: ND 6 Days: 0.09 nM 8 Days: 0.01 nM	4 Days: 4.02 nM 6 Days: 3.40 nM 8 Days: 1.53 nM
NCl-H460	Lung	4 Days: 0.25 nM 6 Days: 0.57 nM 8 Days: 0.56 nM	4 Days: 8.27 nM 6 Days: 5.38 nM 8 Days: 4.77 nM
NCl-H23	Lung	4 Days: 1.00 nM 6 Days: 1.12 nM 8 Days: 0.18 nM	4 Days: ND 6 Days: 7.11 nM 8 Days: 4.78 nM
NCl-H1395	Lung	4 Days: Resistant 6 Days: 6.81 nM 8 Days: 5.27 nM	4 Days: 1009 nM 6 Days: 84.1 nM 8 Days: 15.2 nM
NCl-H2887	Lung	4 Days: Resistant 6 Days: 642.62 nM 8 Days: 6.16 nM	4 Days: Resistant 6 Days: 673.6 nM 8 Days: 9.15 nM
Hep G2	Liver	4 Days: 2.82 nM 6 Days: 0.47 nM 8 Days: 0.63 nM	4 Days: 18.0 nM 6 Days: 1.58 nM 8 Days: 0.98 nM
SK-HEP-1	Liver	4 Days: 5.60 nM 6 Days: 2.85 nM 8 Days: 0.45 nM	4 Days: 39.3 nM 6 Days: 11.2 nM 8 Days: 1.45 nM

Example 7: Peloruside A Effect on Tubulin Polymerization

[0152] Taxol® functions to inhibit cell proliferation through the stabilization of microtubules, reducing the pool of cellular free tubulin. As a result, dividing tumor cells become arrested in mitosis. The present example investigates the capability of Peloruside A to polymerize free tubulin *in vitro*. Synthetic (+)-Peloruside A was used for the present example.

[0153] Tubulin, purified from bovine brains and prepared in PEM-buffer (80 mM PIPES, 0,5 mM $MgCl_2$, 1mM EGTA, pH 6.9) was thawed under vacuum on ice. Tubulin was incubated at 37°C until almost melted and resuspended to a final concentration of 2 mg/ml and placed on ice. Peloruside A or Taxol® was added into transparent 96 – well plates (Corning Incorp. Costar) and placed on ice. GTP (Sigma) was added to a final concentration of 1 mM. The 96 well plate was warmed to 37°C and 120 µl of tubulin solution was added into each well. The absorbance of the reaction at 340 nm over time (each 40 – 50 sec.) was measured on the spectrophotometer TECAN SPECTRAFluor Plus. Peloruside A or Taxol® was added to the tubulin at a final concentration of 5 or 10 µM. The tubulin polymerization curves are shown in Figures 74 and 75.

[0154] Peloruside A demonstrates a potent tubulin stabilization activity (Figure 75). The rate of polymerization is similar to the rate of polymerization exhibited by Taxol® at both 5 or 10 µM of drug (Figure 74). Therefore, Peloruside A may function to inhibit tumor cell growth by disrupting tubulin dynamics.

[0155] In addition, immunostained tubulin in BSC-1 cells show that Peloruside A inhibits the formation of a bipolar spindle (FIG. 97). Control untreated cells exhibit chromosomes lined at the mitotic plate. Similar to Taxol® treatment, the bipolar spindle does not form when BSC-1

cells are treated with Peloruside A. Instead the chromosomes surround the monoaster that forms in the cell.

Example 8: Cytotoxic Effect of Peloruside Analogs

[0156] The cytotoxic effect of synthetic Peloruside A analogs, LX3111 and LX3136, on HeLa and SK-MEL-5 cells was investigated. LX3111 is a compound of the formula II of the present invention and LX3136 is a compound of the formula III of the present invention. Synthetic (+)-Peloruside A analogs were prepared for the present example.

[0157] Cells were treated with medium, DMSO, LX3111 or LX3136 at a final concentration of 10, 50, 100, 200, 300, 400, 500, 600, 800 or 1000 nM. Cell viability was measured by counting surviving cells or measuring luminescence at 48 hours after drug treatment using the Cell Titer-Glo Luminescent Cell Viability Assay (Promega). Figures 76-79 shows bar graphs of the inhibition of cell growth exhibited by the Peloruside analogs.

[0158] Both LX3111 and LX3136 exhibited potent antimitotic activity in inhibiting cell growth of tumor cells, HeLa and SK-MEL-5.

Example 9: Peloruside A Treatment of Taxol®-Resistant Cell Lines

[0159] Taxol®-resistant ovarian cancer cell lines, Ptx-10 and Ptx-22, isolated from the parent 1A9 cell line, contain point mutations in the beta-tubulin gene (Giannakakou et al., Paclitaxel-resistant human ovarian cancer cell have mutant beta-tubulins that exhibit impaired paclitaxel-driven polymerization, J. Biol. Chem., 272, 17118-17125, 1997). These cells were generated by exposure to Taxol® and verapamil and exhibit defective tubulin polymerization. These cell lines have specific amino acid mutations in tubulin that prevent Taxol®-binding. The present

example demonstrates the cytotoxtic effect of Peloruside A treatment on these Taxol®-resistant tumor cell lines in comparison to Taxol® treatment. Synthetic (+)-Peloruside A was used for the present example.

[0160] 1A9, ptx10 and ptx22 cells were plated in 96-well plates to approximately 50% confluency. On the next day, Taxol® or Peloruside A was added to each well and incubated for 48 hours. A final concentration of 1, 2, 3, 4, 5, 8, 10, 20, 30, 50, 100, 300, 500, or 1000 nM of Taxol® was used. A final concentration of 10, 20, 30, 40, 50 or 70 nM of Peloruside A was used. Cell viability was measured using the Cell Titer-Glo Luminescent Cell Viability Assay (Promega). Figures 80-82 show bar graphs of cell viability at various concentrations of Peloruside A and Taxol®.

[0161] Peloruside A demonstrate potent antimitotic activity by inhibiting cell growth of both the parental and Taxol®-resistant tumor cell lines (Figures 80 and 81). Peloruside A reduces cell viability to approximately 50% at 30 nM of drug in ptx10 and ptx22 cells, whereas treatment of these cells must exceed 100 nM to achieve a similar effect with Taxol® treatment. The relative magnitude of the effect caused by Peloruside A treatment suggests a different mechanism of action. Peloruside A may bind either tubulin at the Taxol®-binding site in a manner unaffected by the mutations found in ptx 10 and ptx 22 or Peloruside A binds tubulin at another site. In any case, the experiments demonstrate the utility of Peloruside A in Taxol®-resistant tumor cells.

[0162] All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of the invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the

compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

[0163] Patents, publications, product descriptions and protocols are cited throughout this application, the disclosures of which are incorporated herein by reference in their entireties for all purposes.